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(57) Abstract

The present invention is directed to recombinant hosts expressing novel proteins associated with Alzheimer's Disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas. This invention is specifically directed to the recombinant hosts and vectors which contain the genes coding for the neuronal thread proteins. This invention is also directed to substantially pure neural thread protein, immunodiagnostic and molecular diagnostic methods to detect the presence of neural thread proteins, and the use of nucleic acid sequences which code for neural thread proteins in gene therapy.

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Neural Thread Protein Gene Expression and Detection of Alzheimer's Disease

Field of the Invention

The present invention is in the field of genetic engineering and molecular biology. This invention is directed to recombinant hosts expressing novel proteins associated with Alzheimer's Disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas. This invention is specifically directed to the recombinant hosts and vectors which contain the genes coding for the neuronal thread proteins. This invention is also directed to substantially pure neural thread proteins, immunodiagnostic and molecular diagnostic methods to detect the presence of neural thread proteins, and the use of nucleic acid sequences which code for neural thread proteins in gene therapy.

Background of the Invention

Alzheimer's Disease

Alzheimer's Disease (AD) is the most frequent cause of dementia in the United States, affecting over two million individuals each year. It is a degenerative brain disorder characterized clinically by loss of memory, confusion, and gradual physical deterioration. It is the fourth most common cause of death. The etiology of the disease is virtually unknown but has been attributed to various viruses, toxins, heavy metals, as well as genetic defects. The disease is at present incurable.

Until quite recently, AD was thought to account for relatively few of the cases generally classified as senile dementia. Other factors can lead to such a condition, including repetitious mild strokes, thyroid disorders, alcoholism, and deficiencies of certain vitamins, many of which are potentially treatable. It can be appreciated, then, that a diagnostic test specific for AD would be very useful for the clinical diagnosis and proper clinical treatment of subjects presenting with symptoms common to all of these conditions.

The brains of individuals with AD exhibit characteristic pathological accumulations of congophilic fibrous material which occurs as neurofibrillary tangles within neuronal cell bodies, and neuritic (or senile) plaques. Neurofibrillary tangles may also be found in the walls of certain cerebral blood vessels. The major organized structural components of neurofibrillary tangles are paired helical filaments. Qualitatively indistinguishable amyloid deposits also occur in normal aged brains but in much smaller numbers with restricted topographical distribution.

There has been considerable recent investigative activity regarding the characterization of proteins found in neuritic plaques and neurofibrillary tangles of AD and other neurologic diseases. One of the amyloid proteins initially described by Glenner *et al.* has been cloned and sequenced (Glenner *et al., Biochem. Biophys. Res. Commun. 120*:1131-1135 (1984); U.S. Patent

No. 4,666,829). The A4 amyloid protein found in neuritic plaques and blood vessels has been determined to be a component of a 695 amino acid precursor; a protein postulated to function as a glycosylated cell surface receptor (Masters *et al., Proc. Natl. Acad. Sci. USA 82*:4245-4249 (1985), Kang *et al., Nature 325*:733-736 (1987)). In addition, the amyloid protein has been postulated to function as a cell adhesion molecule and as a calcium ion channel protein (Hooper, *J. NIH Res. 4*: 48-54 (1992); Rensberger, *Wayward Protein Molecule May Be Elusive Killer of Brain Cells, The Washington Post*, January 25, 1993, §1, at A3 (1993)). The gene coding for A4 is located on chromosome 21 (Kang *et al., ibid.*; Goldgaber *et al., Science 235*:877-880 (1987); Tanzi *et al., Science 235*:880-885 (1987); St. George-Hyslop *et al., Science 235*:885-889 (1987)) but apparently is not linked to the familial form of the disease (Van Broekhoven *et al., Nature 329*:153-155 (1987)). There appears to be little, if any, protein sequence homology between amyloid A4 and ß protein, their higher molecular weight precursor, and pancreatic thread protein (PTP) (Gross *et al., J. Clin. Invest. 76*:2115-2126 (1985)).

A number of other proteins thought to be associated with the disease have been described, including Ubiquitin, ALZ-50, microtubular-associated proteins τ and MAP2, and neurofilament protein (*see*, for example, Manetto *et al., Proc. Natl. Acad. Sci. USA 85*:4502-4505 (1988); Wolozin *et al., Science 232*:648-651 (1986); Selkoe, *Neurobiol. Aging 7*:425-432 (1986); Perry *et al.*, in: *Alterations of the Neuronal Cytoskeleton in Alzheimer's Disease*, Plenum, New York, pp 137-149 (1987)). More recently, a serine protease inhibitor called α_1-anti-chymotrypsin has been found in AD amyloid deposits (Abraham *et al., Cell 52*:487-501 (1988)).

There is currently no useful diagnostic test for AD being practiced clinically. A definitive diagnosis is possible only postmortem, or during life through a brain biopsy, to reveal the presence of the characteristic plaques, tangles, paired helical filaments, and other cerebrovascular deposits which characterize the disorder. Such an invasive surgical procedure is inherently

dangerous and is therefore rarely utilized. As a result, the clinical misdiagnosis of AD is estimated to be approximately 20%-30%.

Thread Proteins

The prototype thread protein molecule is pancreatic thread protein (PTP) which bears the unusual physical property of forming insoluble fibrils at neutral pH, but is highly soluble at acid or alkaline pH (Gross *et al.*, *supra*). PTP is highly abundant, synthesized by pancreatic acinar cells, and secreted into pancreatic juice in concentrations exceeding 1 mg/ml (*Id.*). An increased thread protein immunoreactivity has been demonstrated in brains with AD lesions, using monoclonal antibodies to PTP (Ozturk *et al.*, *Proc. Natl. Acad. Sci. USA 86*:419-423 (1989)). In addition, a highly sensitive forward sandwich immunoradiometric assay was used to demonstrate that at least three distinct antigenic epitopes were shared between PTP and the related protein in the brain (*Id.*) Despite similarities, the pancreatic and neuronal forms of the thread protein are almost certainly distinct since the mRNA molecules and proteins differ in size, and many of the antigenic epitopes which are present in the pancreatic thread protein are not detectable in brain tissue (de la Monte *et. al.*, *J. Clin. Invest. 86*:1004-1013 (1990); de la Monte *et. al.*, *J. Neurol. Sci. 113*:152-164 (1992); de la Monte *et al.*, *Ann. Neurol. 32*:733-742 (1992)).

The central nervous system form of the thread protein, designated hereafter as "neural thread protein" (NTP), has been identified in AD and Down's Syndrome brain tissue (Wands *et al.*, International Application Publication No. WO 90/06993). NTP has been found in all AD brains studied where characteristic neuropathologic changes of the disease exist (*Id.*). The saline- extractable soluble immunoreactivity shares has a molecular weight of approximately 17 to 20 kD (*Id.*).

Quantitative measurements of NTP immunoreactivity in various regions of AD brains revealed levels varying from 12 to 295 ng/gm tissue (Mean =

116 ng/gm tissue) compared to 1-11 ng/gm tissue (Mean = 5 ng/gm tissue) in comparable ares of control brains (*Id.*).

Immunocytochemistry performed with monoclonal antibodies directed against the pancreatic form of PTP demonstrated that NTP is localized within cells, within fine processes within the neuropil, or is extracellular in both AD and Down's Syndrome brains (*Id.*). Two types of cell contain NTP: neurons and astrocytes (*Id.*). The affected neurons are the large pyramidal type which typically contain the neurofibrillary tangles well known in AD brain (*Id.*).

That NTP accumulation within neurons is intrinsically important or integrally related to the evolution of AD lesions is corroborated by the presence of identical patterns of immunolabeling for NTP in Down's Syndrome brains, but not in control brains (*Id.*). It is important to note that the same structural abnormalities of AD occur in brains of all middle-age individuals with Down's syndrome, whether or not they are demented. There is also a higher incidence of AD in family members of Down's Syndrome patients. Moreover, the regional differences in the densities of NTP-containing neurons parallels the density distributions of neurofibrillary tangles in both AD and Down's Syndrome. This provides further evidence that NTP is germane to the pathophysiology of AD. Whether NTP accumulates within neuronal perikarya, as a result of aberrant cellular metabolism or transport is not yet known.

Summary of the Invention

A need exists for a definitive diagnostic test which can be performed on individuals suspected of having, or being at risk for AD. The present invention satisfies such needs and provides further advantages.

The manner in which these and other objects are realized by the present invention will be apparent from the summary and detailed description set forth below.

Unless defined otherwise, various terms used herein have the same meaning as is well understood in the art to which the invention belongs. All cited publications are incorporated herein by reference.

This invention is directed to recombinant hosts expressing novel proteins associated with Alzheimer's Disease, neuroectodermal tumors, malignant astrocytomas, and glioblastomas. This invention is specifically directed to the recombinant hosts and vectors which contain the genes coding for the neuronal thread proteins (NTP) having molecular weights of about 8 kDa, 14 kDa, 17 kDa, 21 kDa, 26 kDa or 42 kDa. This invention is also directed to the substantially pure neural thread proteins, immunodiagnostic and molecular diagnostic methods to detect the presence of neural thread proteins, and the use of nucleic acid sequences which code for neural thread proteins in gene therapy.

In particular, the invention includes a method for detecting and quantitating an NTP in a human subject, comprising:

(a) contacting a biological sample from a human subject that is suspected of containing detectable levels of an NTP with a molecule capable of binding to the NTP; and

(b) detecting the molecule bound to the NTP.

The invention additionally includes the method as above, wherein the binding molecule is selected from the group consisting of:

(a) an antibody substantially free of natural impurities;

(b) a monoclonal antibody; and

(c) a fragment of (a) or (b).

The invention additionally includes the method as above, wherein the detecting molecule is detectably labeled and where a combination of such binding molecules is used.

The invention additionally includes a method for detecting the presence of a genetic sequence coding for an NTP in a biological sample using a polynucleotide probe derived from a recombinant human NTP of this invention.

The invention additionally includes a method for determining the presence of a condition in a human subject, said condition including, but not limited to, the group consisting of Alzheimer's Disease, the presence of neuroectodermal tumors, the presence of malignant astrocytomas, and the presence of gliomas.

The invention additionally includes a method of diagnosing the presence of AD in a human subject suspected of having AD which comprises:

(a) incubating a biological sample from said subject suspected of containing an NTP with a molecule capable of identifying an NTP; and

(b) detecting the molecule which is bound in the sample, wherein the detection indicates that the subject has AD.

The invention additionally includes a method of diagnosing the presence of neuroectodermal tumors in a human subject suspected of having neuroectodermal tumors which comprises:

(a) incubating a biological sample from said subject suspected of containing an NTP with a molecule capable of identifying an NTP; and

(b) detecting the molecule which is bound in the sample, wherein the detection indicates that the subject has neuroectodermal tumors.

The invention additionally includes a method of diagnosing the presence of a malignant astrocytoma in a human subject suspected of having a malignant astrocytoma which comprises:

(a) incubating a biological sample from said subject, which is suspected of containing an NTP, in the presence of a binding molecule capable of identifying an NTP; and

(b) detecting molecule which is bound in the sample, wherein the detection indicates that the subject has a malignant astrocytoma.

The invention additionally includes a method of diagnosing the presence of a glioblastoma in a human subject suspected of having a glioblastoma which comprises:

(a) incubating a biological sample from said subject, which is suspected of containing an NTP, in the presence of a binding molecule capable of identifying an NTP; and

(b) detecting molecule which is bound in the sample, wherein the detection indicates that the subject has a glioblastoma.

The invention additionally includes the methods as above, wherein a biological sample is removed a human subject prior to contacting the sample with the molecule.

The invention additionally includes the methods as above, wherein detecting any of the molecules bound to the protein is performed by *in situ* imaging.

The invention additionally includes the methods as above, wherein detecting of any of the molecule bound to the protein is performed by *in vivo* imaging.

The invention additionally includes the methods as above, wherein the biological sample is reacted with the binding molecule in a manner and under such conditions sufficient to determine the presence and the distribution of the protein.

The invention additionally includes the methods as above, wherein a detectably labeled binding molecule of an NTP is administered to a human subject.

The invention additionally includes the methods as above, wherein the binding molecule is bound to the protein *in vivo*.

The invention additionally involves an NTP substantially free of any natural impurities and having a molecular weight of about 42 kDa.

The invention additionally involves an NTP substantially free of any natural impurities and having a molecular weight of about 26 kDa.

The invention additionally includes an NTP substantially free of any natural impurities and having a molecular weight of about 21 kDa.

The invention additionally includes an NTP substantially free of any natural impurities and having a molecular weight of about 17 kDa.

The invention additionally includes an NTP substantially free of any natural impurities and having a molecular weight of about 14 kDa.

The invention additionally includes an NTP substantially free of any natural impurities and having a molecular weight of about 8 kDa.

The present invention also particularly relates to the diagnostic methods recited above, wherein the immunoassay comprises two different antibodies bound to a solid phase support combined with a third different detectably labeled antibody in solution.

The invention is also directed to a method of producing an NTP, said method comprising:

(a) culturing a recombinant host comprising a human gene coding for said NTP; and

(b) isolating said NTP from said host.

Additionally, the invention is directed to a substantially pure NTP obtained by the such a process.

The invention is also directed to an 15- to 30-mer antisense oligonucleotide which is complementary to an NTP nucleic acid sequence and which is nonhomologous to PTP nucleic acid sequences, as well as pharmaceutical compositions comprising such oligonucleotides and a pharmaceutically acceptable carrier.

The invention is also directed to ribozymes comprising a target sequence which is complementary to an NTP sequence and nonhomologous to PTP nucleic acid sequences, as well as pharmaceutical compositions comprising such ribozymes and a pharmaceutically acceptable carrier.

The invention is also directed to a method of achieving pharmaceutical delivery of NTP molecules to the brain through acceptable carriers or expression vectors.

The invention is also directed to oligodeoxynucleotides that form triple stranded regions with the various NTP genes (nucleic acid sequences) and

which are nonhomologous to PTP nucleic acid sequences, as well as pharmaceutical compositions comprising such oligodeoxynucleotides and a pharmaceutically acceptable carrier.

The invention is also directed to the therapeutic use of NTP-derived molecules or fragments thereof to modify or improve dementias of the Alzheimer's type of neuronal degeneration.

The invention is also directed to methods for the differential diagnosis of sporadic and familial Alzheimer's disease.

Brief Description of the Drawings

Figures 1A-1J show neural thread protein immunoreactivity in CNS-derived tumors.

Figure 2 depicts a graph showing neural thread protein levels in PNET1, PNET2, A172, C6, and Huh7 hepatocellular carcinoma cells measured by a forward sandwich monoclonal antibody-based immunoradiometric assay (M-IRMA).

Figure 3 shows molecular size of neural thread proteins in SH-Sy5y, A172, and C6 cells demonstrated by immunoprecipitation and Western blot analysis using the Th9 monoclonal antibody.

Figure 4 shows molecular sizes of neural thread proteins in PNET1 cells (a) and C6 glioblastoma cells (b) demonstrated by pulse-chase metabolic labeling with ^{35}S-methionine, and immunoprecipitation with Th9 monoclonal antibody (Figure 4A). The molecular weights are 8, 14, 17, 21, 26 and 42 kDa (arrows).

Figures 5A-5E depict a series of five graphs showing the 21 kDa and 17 kDa neural thread proteins in SH-Sy5y, PNET1, A172, and C6 cells and the absence thereof in Huh7 cells by SDS-PAGE/M-IRMA.

Figure 6 depicts a gel showing that the 21 kDa neural thread protein in C6 glioblastoma cells is phosphorylated.

Figure 7 depicts a bar graph showing altered neural thread protein expression in PNET1 cells with growth phase.

Figures 8A-8F show altered phenotype of PNET1 cells with cessation of cell growth and overnight serum starvation.

Figure 9 shows the 1-9a partial cDNA sequence, and Figure 9A shows a partial sequence of the second 5' anchor PCR product corresponding to the 5' region of the 1-9a cDNA (WP5' Sequence).

Figure 10 shows alignment of partial sequences between 1-9a and human PTP and the Reg gene (the nucleic acid sequence corresponding to the genomic clone of human PTP).

Figure 10A shows alignment between 1-9a and Exon 2 of the human Reg gene, and between the first 5' anchor PCR product of 1-9a (WP03-417) and Exon 2 and Reg.

Figure 10B shows alignment between the 1-9a and its second 5' anchor PCR product (WP5') and AD 3-4 and AD 2-2 cDNAs.

Figure 11A shows the partial nucleic acid and deduced amino acid sequences of the HB4 cDNA. Figures 11B and 11C show a protein hydrophilicity window plot. Hydrophilicity Window Size = 7; scale = Kyte-Doolittle.

Figure 11D shows alignment between HB4 and human PTP.

Figure 11E shows alignment between HB4 and human Reg gene.

Figures 12A-12C show the expression of mRNA molecules corresponding to the 1-9a CNS neural thread protein cDNA sequence in neuroectodermal tumor cell lines and in rat pancreas.

Figures 13A and 13B show mRNA transcripts corresponding to the 1-9a CNS neural thread protein cDNA sequence in human brain. This figure also demonstrates higher levels of 1-9a CNS neural thread protein-related mRNAs in AD brains compared with aged-matched controls (Figure 13A). Figure 13B demonstrates four different transcripts with greater abundance of the lower molecular size mRNAs in AD compared with aged controls.

Figures 14A-14C show 1-9a Southern blot analysis of RT/PCR-derived cDNAs in neuroectodermal cell lines. A- and B-PCR amplification of 1-9a mRNA sequences in neuroectodermal cell lines, and using mRNA from newborn rat (NB) brain, AD brain, and aged control brain. Figure 14A is a longer exposure of Figure 14B. Figure 14C shows hybridization of the same blot using the O18 rat PTP probe.

Figures 15A and 15B (SE-RT/PCR) show hybridization of the 1-9a and O18 probes with several clones isolated from SH-Sy5y cells by reverse transcribing mRNA and amplifying with primers corresponding to the known sequence of the 1-9a partial cDNA.

Figures 16A, 16D and 16E show the partial nucleic acid sequences of the AD 2-2 cDNAs isolated from the AD brain library. Figures 16B and 16C show a hydrophilicity window plot of AD2-2 T7. Hydrophilicity Window Size = 7; scale = Kyte-Doolittle.

Figures 16F, 16I, 16J and 16K show the partial nucleic acid sequences of the AD 3-4 cDNAs isolated from the AD brain library. Figures 16G and 16H show a hydrophilicity window plot of AD3-4. Hydrophilicity Window Size = 7; scale = Kyte-Doolittle.

Figures 16L, 16M and 16N show the partial nucleic acid sequences of the AD 4-4 cDNAs isolated from the AD brain library.

Figure 16O shows the partial nucleic acid sequences of the AD 16c (also called AD 10-7) cDNAs isolated from the AD brain library. Figures 16P and 16Q show a hydrophilicity window plot of AD16c-T7. Hydrophilicity Window Size = 7; scale = Kyte-Doolittle.

Figure 16R shows the complete nucleotide sequence of the AD10-7 cDNA clone that was isolated from an AD library.

Figure 16S shows the complete nucleotide sequence of the AD16c cDNA clone that was isolated from the AD brain library.

Figure 17 shows alignment of partial sequences between AD 2-2 and human Reg gene.

Figure 17A shows alignment of partial sequences between AD 2-2 and Exon 1 of Reg and rat PTP.

Figure 17B shows alignment of partial sequences between AD 2-2 and 1-9a.

Figure 17C shows alignment of partial sequences between AD 2-2 and AD 16c.

Figure 18 shows alignment of partial sequences between AD 3-4 (also called AD 5-3) and the Reg gene.

Figure 18A shows alignment of partial sequences between AD 3-4 and the 5' anchor PCR products of the 1-9a mRNA, termed WPO3-5 and 18-4.

Figure 18B shows alignment of partial sequences between AD 3-4 and the G2a-a *Eco*RI/*Pst*I genomic clone.

Figure 19 shows alignment of partial sequences between AD 4-4 and AD 2-2 and 1-9a (also called SE-4 corresponding to the PCR clone which is identical to 1-9a).

Figure 20 shows alignment of partial sequences between AD 16c and Reg gene.

Figure 20A shows alignment of partial sequences between AD 16c and human PTP.

Figure 20B shows alignment of partial sequences between AD 16c and AD 2-2.

Figures 21A-21D show a genomic Southern blot analysis using the AD 3-4 as a probe; Figure 21B shows a similar pattern of hybridization on a genomic Southern using AD 2-2 as a probe. Figures 21A-21D show a Northern blot analysis of neuroectodermal tumor cell lines using AD 3-4 as a probe. The four cell lines that exhibit AD 3-4 transcripts are neuronal in phenotype; C6 glioma cell mRNA did not hybridize with the AD 3-4 probe. Figure 21D shows a Northern analysis of human AD and aged control brain temporal lobe tissue using the AD 3-4 probe, and demonstrates over-expression of the corresponding gene in AD (lanes labeled A) compared with aged control brains (lanes labeled C).

Figures 22, 22A, 22B, 22C, 22D, 22E, 22F, 22G and 22H show partial sequences of four genomic clones (isolated using both the 1-9a cDNA and rat PTP O-18 cDNA as probes.

Figures 23 and 23A show the alignment of the G2a-2 *Pst*I partial sequence with the Reg gene.

Figure 23B shows alignment of the G2a-2 *Pst*I-*Eco*RI sequence and the Reg gene and the rat PTP.

Figures 23C and 23D show the alignment of the G5d-1 *Pst*I sequence and the Reg gene.

Figures 24A-24D show neural thread protein expression by the 1-9a cDNA (Figure 24A) and the G2a-2 *Pst*I genomic clone (Figure 24B). Figures 24C and 24D show negative expression by the G5d-1 *Eco*RI/*Pst*I genomic clone, and pBluescript which lacks a cloned insert, respectively.

Figures 25A and 25B depict a Northern blot analysis of AD16c mRNA in AD and aged control brains. The data shows elevated levels of AD16c mRNA expression in 6 of 9 AD compared to 1 of 6 age-matched controls.

Figure 26 depicts a Western blot analysis of AD10-7 fusion proteins using monoclonal antibodies against the expressed tag protein (T7-tag mouse monoclonal antibodies.

Figures 27A and 27B depict brightfield and darkfield microscopic analysis of the *in situ* hybridization of sense and antisense cRNA probes to human brain tissue sections of early AD.

Definitions

In the description that follows, a number of terms used in recombinant DNA technology are utilized extensively. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

Cloning vector. A plasmid or phage DNA or other DNA sequence which is able to replicate autonomously in a host cell, and which is

characterized by one or a small number of restriction endonuclease recognition sites at which such DNA sequences may be cut in a determinable fashion without loss of an essential biological function of the vector, and into which a DNA fragment may be spliced in order to bring about its replication and cloning. The cloning vector may further contain a marker suitable for use in the identification of cells transformed with the cloning vector. Markers, for example, provide tetracycline resistance or ampicillin resistance.

Expression vector. A vector similar to a cloning vector but which is capable of enhancing the expression of a gene which has been cloned into it, after transformation into a host. The cloned gene is usually placed under the control of (i.e., operably linked to) certain control sequences such as promoter sequences. Promoter sequences may be either constitutive or inducible.

Substantially pure. As used herein means that the desired purified protein is essentially free from contaminating cellular components, said components being associated with the desired protein in nature, as evidenced by a single band following polyacrylamide-sodium dodecyl sulfate gel electrophoresis. Contaminating cellular components may include, but are not limited to, proteinaceous, carbohydrate, or lipid impurities.

The term "substantially pure" is further meant to describe a molecule which is homogeneous by one or more purity or homogeneity characteristics used by those of skill in the art. For example, a substantially pure NTP will show constant and reproducible characteristics within standard experimental deviations for parameters such as the following: molecular weight, chromatographic migration, amino acid composition, amino acid sequence, blocked or unblocked N-terminus, HPLC elution profile, biological activity, and other such parameters. The term, however, is not meant to exclude artificial or synthetic mixtures of the factor with other compounds. In addition, the term is not meant to exclude NTP fusion proteins isolated from a recombinant host.

Recombinant Host. According to the invention, a recombinant host may be any prokaryotic or eukaryotic cell which contains the desired cloned

genes on an expression vector or cloning vector. This term is also meant to include those prokaryotic or eukaryotic cells that have been genetically engineered to contain the desired gene(s) in the chromosome or genome of that organism.

Recombinant vector. Any cloning vector or expression vector which contains the desired cloned gene(s).

Host. Any prokaryotic or eukaryotic cell that is the recipient of a replicable expression vector or cloning vector. A "host," as the term is used herein, also includes prokaryotic or eukaryotic cells that can be genetically engineered by well known techniques to contain desired gene(s) on its chromosome or genome. For examples of such hosts, *see* Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989).

Promoter. A DNA sequence generally described as the 5′ region of a gene, located proximal to the start codon. The transcription of an adjacent gene(s) is initiated at the promoter region. If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter.

Gene. A DNA sequence that contains information needed for expressing a polypeptide or protein.

Structural gene. A DNA sequence that is transcribed into messenger RNA (mRNA) that is then translated into a sequence of amino acids characteristic of a specific polypeptide.

Antisense RNA gene/Antisense RNA. In eukaryotes, mRNA is transcribed by RNA polymerase II. However, it is also known that one may construct a gene containing a RNA polymerase II template wherein a RNA sequence is transcribed which has a sequence complementary to that f a specific mRNA but is not normally translated. Such a gene construct is herein termed an "antisense RNA gene" and such a RNA transcript is termed an

"antisense RNA." Antisense RNAs are not normally translatable due to the presence of translation stop codons in the antisense RNA sequence.

Antisense oligonucleotide. A DNA or RNA molecule containing a nucleotide sequence which is complementary to that of a specific mRNA. An antisense oligonucleotide binds to the complementary sequence in a specific mRNA and inhibits translation of the mRNA.

Antisense Therapy. A method of treatment wherein antisense oligonucleotides are administered to a patient in order to inhibit the expression of the corresponding protein.

Complementary DNA (cDNA). A "complementary DNA," or "cDNA" gene includes recombinant genes synthesized by reverse transcription of mRNA and from which intervening sequences (introns) have been removed.

Expression. Expression is the process by which a polypeptide is produced from a structural gene. The process involves transcription of the gene into mRNA and the translation of such mRNA into polypeptide(s).

Homologous/Nonhomologous Two nucleic acid molecules are considered to be "homologous" if their nucleotide sequences share a similarity of greater than 50%, as determined by HASH-coding algorithms (Wilber, W.J. and Lipman, D.J., *Proc. Natl. Acad. Sci. 80*:726-730 (1983)). Two nucleic acid molecules are considered to be "nonhomologous" if their nucleotide sequences share a similarity of less than 50%.

Ribozyme. A ribozyme is an RNA molecule that contains a catalytic center. The term includes RNA enzymes, self-splicing RNAs, and self-cleaving RNAs.

Ribozyme Therapy. A method of treatment wherein ribozyme is administered to a patient in order to inhibit the translation of the target mRNA.

Fragment. A "fragment" f a molecule such as NTP is meant to refer to any polypeptide subset of that molecule.

Functional Derivative. The term "functional derivatives" is intended to include the "variants," "analogues," or "chemical derivatives" of the

molecule. A "variant" of a molecule such as NTP is meant to refer to a naturally occurring molecule substantially similar to either the entire molecule, or a fragment thereof. An "analogue" of a molecule such as NTP is meant to refer to a non-natural molecule substantially similar to either the entire molecule or a fragment thereof.

A molecule is said to be "substantially similar" to another molecule if the sequence of amino acids in both molecules is substantially the same, and if both molecules possess a similar biological activity. Thus, provided that two molecules possess a similar activity, they are considered variants as that term is used herein even if one of the molecules contains additional amino acid residues not found in the other, or if the sequence of amino acid residues is not identical.

As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half-life, etc. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Examples of moieties capable of mediating such effects are disclosed in *Remington's Pharmaceutical Sciences* (1980) and will be apparent to those of ordinary skill in the art.

NTP. The term "NTP" refers to a family of neural thread proteins. The NTP family includes proteins with molecular weights of about 8 kDa, 14 kDa, 17 kDa, 21 kDa, 26 kDa and 42 kDa, as described herein.

Immuno-Polymerase Chain Reaction. A method for the detection of antigens using specific antibody-DNA conjugates. According to this method, a linker molecule with bispecific binding affinity for DNA and antibodies is used to attach a DNA molecule specifically to an antigen-antibody complex. As a result, a specific antigen-antibody-DNA conjugate is formed. The attached DNA can be amplified by the polymerase chain reaction (PCR) using appropriate oligonucleotide primers. The presence of specific PCR products demonstrates that DNA molecules are attached specifically to antigen-antibody

complexes, thus indicating the presence of antigen. (Sano *et al., Science 258*:120-122 (1992)).

For example, Sano *et al., supra*, constructed a streptavidin-protein A chimera that possesses specific binding affinity for biotin and immunoglobulin G. This chimera (i.e., the "linker molecule") was used to attach a biotinylated DNA specifically to antigen-monoclonal antibody complexes that had been immobilized on microtiter plate wells. A segment of the attached DNA was subsequently amplified by PCR.

Detailed Description of the Invention

This invention is directed to neural thread proteins (NTP), genetic sequences coding for an NTP mRNA or antisense mRNA, expression vectors containing the genetic sequences, recombinant hosts transformed therewith, and NTP and antisense RNA produced by such transformed recombinant host expression. This invention further relates to NTP ribozymes, and recombinant DNA molecules which code for NTP ribozymes and NTP antisense oligonucleotides. This invention further relates to antibodies directed against an NTP, as well as the use of NTP antibodies and NTP nucleic acid sequences for detection of the presence of an NTP in biological samples. The invention further relates to the use of NTP coding sequences in gene therapy.

I. Isolation of DNA Sequences Coding for Neuronal Thread Proteins

DNA sequences coding for an NTP are derived from a variety of sources. These sources include genomic DNA, cDNA, synthetic DNA, and combinations thereof.

Human NTP genomic DNA can be extracted and purified from any human cell or tissue, by means well known in the art (for example, *see* Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989). The NTP genomic DNA of the

invention may or may not include naturally occurring introns. Moreover, such genomic DNA may be obtained in association with the 5′ promoter region of the NTP gene sequences and/or with the 3′ translational termination region. Further, such genomic DNA may be obtained in association with DNA sequences which encode the 5′ nontranslated region of the NTP mRNA and/or with the genetic sequences which encode the 3′ nontranslated region. To the extent that a host cell can recognize the transcriptional and/or translational regulatory signals associated with the expression of the mRNA and protein, then the 5′ and/or 3′ nontranscribed regions of the native gene, and/or, the 5′ and/or 3′ nontranslated regions of the mRNA, may be retained and employed for transcriptional and translational regulation.

Alternatively, an NTP mRNA can be isolated from any cell which expresses an NTP, and used to produce cDNA by means well known in the art (for example, *see* Sambrook *et al., supra*). Preferably, the mRNA preparation used will be enriched in mRNA coding for an NTP, either naturally, by isolation from cells which produce large amounts of an NTP, or *in vitro*, by techniques commonly used to enrich mRNA preparations for specific sequences, such as sucrose gradient centrifugation, or both. An NTP mRNA may be obtained from mammalian neuronal tissue, or from cell lines derived therefrom. Preferably, human cDNA libraries are constructed from 17-18 week old fetal brain, 2 year old temporal lobe neocortex, end-stage AD cerebral cortex, or from cell lines derived from human neuronal tissue. Such cell lines may include, but are not limited to, central nervous system primitive neuroectodermal tumor cells (such as PNET1 or PNET2, as described herein), neuroblastoma cells (such as SH-Sy5y, as described herein), or human glioma cells (such as A172; ATCC CRL 1620). Alternatively, a rat cDNA library can be prepared from mRNA isolated from rat glioma cells, for example, C6 rat glioma cells (ATCC CCL107).

For cloning into a vector, suitable DNA preparations (either genomic or cDNA) are randomly sheared or enzymatically cleaved, respectively, and ligated into appropriate vectors to form a recombinant gene (either genomic

or cDNA) library. A DNA sequence encoding an NTP may be inserted into a vector in accordance with conventional techniques, including blunt-ending or staggered-ending termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulation are disclosed by Sambrook *et al.*, *supra*, and are well known in the art.

Libraries containing NTP clones may be screened and the NTP clones identified by any means which specifically selects for NTP DNA such as, for example: 1) by hybridization with an appropriate nucleic acid probe(s) containing a sequence specific for the DNA of this protein; or, 2) by hybridization-selected translational analysis in which native mRNA hybridizes to the clone in question, is translated *in vitro*, and the translation products are further characterized; or, 3) if the cloned DNA sequences are themselves capable of expressing mRNA, by immunoprecipitation of a translated NTP product produced by the host containing the clone.

Oligonucleotide probes specific for an NTP which can be used to identify clones to this protein can be designed from knowledge of the amino acid sequence of the corresponding NTP, or homologous regions of the PTP. Alternatively, oligonucleotide probes can be designed from knowledge of the nucleotide sequence of PTP (de la Monte *et al.*, *J. Clin. Invest.* *86*:1004-1013 (1990)).

The suitable oligonucleotide, or set of oligonucleotides, which is capable of encoding a fragment of the NTP gene (or which is complementary to such an oligonucleotide, or set of oligonucleotides) may be synthesized by means well known in the art (for example, *see* Sambrook *et al.*, *supra*). Techniques of nucleic acid hybridization and clone identification are disclosed by Sambrook *et al.*, *supra*. Those members of the above-described gene library which are found to be capable of such hybridization are then analyzed to determine the extent and nature of the NTP encoding sequences which they contain.

To facilitate the detection of the desired NTP coding sequence, the above-described DNA probe is labeled with a detectable group. Such detectable group can be any material having a detectable physical or chemical property. Such materials have been well-developed in the field of nucleic acid hybridization and in general most any label useful in such methods can be applied to the present invention. Particularly useful are radioactive labels including ^{32}P, ^{3}H, ^{14}C, ^{125}I, or the like. Any radioactive label may be employed which provides for an adequate signal and has sufficient half-life. The DNA probe may be labeled, for example, by nick-translation, by T4 DNA polymerase replacement synthesis, or by random priming, among other methods well known in the art (*see* Sambrook *et al. supra*).

Alternatively, DNA probes can be labeled with non-radioactive markers such as biotin, an enzyme, or fluorescent group.

In an alternative method of cloning NTP DNA sequences, NTP cDNAs are obtained by direct cloning of cDNAs from cell lines and brain tissue, using the 3'- and 5'-RACE methods, as described herein. Preferably, a human neuroectodermal tumor cell line or AD brain tissue is used as a source of mRNA.

II. Expressing the Gene Coding for NTP

The above-discussed methods are, therefore, capable of identifying DNA sequences which are code for an NTP or fragments thereof. In order to further characterize such DNA sequences, and in order to produce the recombinant protein, it is desirable to express the proteins which the DNA sequences encode.

To express an NTP, transcriptional and translational signals recognizable by an appropriate host are necessary. The cloned NTP DNA sequences, obtained through the methods described above, and preferably in double-stranded form, may be "operably linked" to sequences controlling transcriptional expression in an expression vector, and introduced into a host

cell, either prokaryotic or eukaryotic, to produce recombinant NTP. Depending upon which strand of the NTP coding sequence is operably linked to the sequences controlling transcriptional expression, it is also possible to express an NTP antisense RNA.

Expression of the NTP in different hosts may result in different post-translational modifications which may alter the properties of the NTP. Preferably, the present invention encompasses the expression of an NTP in eukaryotic cells, and especially mammalian, insect, and yeast cells. Especially preferred eukaryotic hosts are mammalian cells. Mammalian cells provide post-translational modifications to recombinant NTP which include folding and/or phosphorylation. Most preferably, mammalian host cells include human CNS primitive neuroectodermal tumor cells, human neuroblastoma cells, human glioma cells, or rat glioma cells. Especially preferred primitive neuroectodermal tumor cells include PNET1 and PNET2, especially preferred human glioblastoma cells include Hg16 and Hg17, especially preferred human glioma cells include A172, and especially preferred rat glioma cells include C6 (*see* Example 1).

Alternatively, an NTP may be expressed by prokaryotic host cells. Preferably, a recombinant NTP is expressed by such cells as a fusion protein, as described herein. An especially preferred prokaryotic host is *E. coli*. Preferred strains of *E. coli* include Y1088, Y1089, CSH18, ER1451, and ER1647 (*see*, for example, *Molecular Biology LabFax*, Brown, T.A., Ed., Academic Press, New York (1991)). An alternative preferred host is *Bacillus subtilus*, including such strains as BR151, YB886, MI119, MI120, and B170 (*see*, for example, Hardy, "Bacillus Cloning Methods," in *DNA Cloning: A Practical Approach*, IRL Press, Washington, D.C. (1985)).

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains expression control sequences which in turn contain transcriptional regulatory information and such sequences are "operably linked" to the nucleotide sequence which encodes the protein.

Two sequences of a nucleic acid molecule are said to be operably linked when they are linked to each other in a manner which either permits both sequences to be transcribed onto the same RNA transcript, or permits an RNA transcript, begun in one sequence to be extended into the second sequence. Thus, two sequences, such as a promoter sequence and any other "second" sequence of DNA or RNA are operably linked if transcription commencing in the promoter sequence will produce an RNA transcript of the operably linked second sequence. In order to be operably linked it is not necessary that two sequences be immediately adjacent to one another.

The promoter sequences of the present invention may be either prokaryotic, eukaryotic or viral. Suitable promoters are repressible, constitutive, or inducible. Examples of suitable prokaryotic promoters include promoters capable of recognizing the T4 polymerases (Malik *et al., J. Biol. Chem. 263*:1174-1181 (1984); Rosenberg *et al., Gene 59*:191-200 (1987); Shinedling *et al., J. Molec. Biol. 195*:471-480 (1987); Hu *et al., Gene 42*:21-30 (1986)), T3, Sp6, and T7 (Chamberlin *et al., Nature 228*:227-231 (1970); Bailey *et al., Proc. Natl. Acad. Sci. (U.S.A.) 80*:2814-2818 (1983); Davanloo *et al., Proc. Natl. Acad. Sci. (U.S.A.) 81*:2035-2039 (1984)); the P_R and P_L promoters of bacteriophage lambda (*The Bacteriophage Lambda*, Hershey, A.D., Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1973); *Lambda II*, Hendrix, R.W., Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1980)); the trp, recA, heat shock, and lacZ promoters of *E. coli*; the α-amylase (Ulmanen *et al., J. Bacteriol. 162*:176-182 (1985)) and the delta-28-specific promoters of *B. subtilis* (Gilman *et al., Gene 32*:11-20 (1984)); the promoters of the bacteriophages of *Bacillus* (Gryczan, In: *The Molecular Biology of the Bacilli*, Academic Press, Inc., NY (1982)); *Streptomyces* promoters (Ward *et al., Mol. Gen. Genet. 203*:468-478 (1986)); the *int* promoter of bacteriophage lambda; the *bla* promoter of the ß-lactamase gene of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene of pBR325, etc. Prokaryotic promoters are reviewed by Glick, *J. Ind. Microbiol. 1*:277-282 (1987); Cenatiempo, *Biochimie 68*:505-516

(1986); Watson *et al.*, In: *Molecular Biology of the Gene*, Fourth Edition, Benjamin Cummins, Menlo Park, CA (1987); Gottesman, *Ann. Rev. Genet. 18*:415-442 (1984); and Sambrook *et al., supra.*

Preferred eukaryotic promoters include the promoter of the mouse metallothionein I gene (Hamer *et al., J. Mol. Appl. Gen. 1*:273-288 (1982)); the TK promoter of Herpes virus (McKnight, *Cell 31*:355-365 (1982)); the SV40 early promoter (Benoist, *et al., Nature (London) 290*:304-310 (1981)); and the yeast *gal4* gene promoter (Johnston, *et al., Proc. Natl. Acad. Sci. (USA) 79*:6971-6975 (1982); Silver, *et al., Proc. Natl. Acad. Sci. (USA) 81*:5951-5955 (1984)). All of the above listed references are incorporated by reference herein.

Strong promoters are the most preferred promoters of the present invention. Examples of such preferred promoters are those which recognize the T3, SP6 and T7 polymerase promoters; the P_L promoter of bacteriophage lambda; the *recA* promoter and the promoter of the mouse metallothionein I gene. The most preferred promoter for expression in prokaryotic cells is one which is capable of recognizing the T7 polymerase promoter. The sequences of such polymerase recognition sequences are disclosed by Watson, *et al.* (In: *Molecular Biology of the Gene*, Fourth Edition, Benjamin Cummins, Menlo Park, CA, (1987)). The most preferred promoter for expression in mammalian cells is SV40 (Gorman, "High Efficiency Gene Transfer into Mammalian cells," in *DNA Cloning: A Practical Approach*, Volume II, IRL Press, Washington, D.C., pp. 143-190 (1985)).

III. Methods of Detecting NTP

This invention is directed towards methods of detecting neurological disease in a human subject, utilizing the nucleic acid probes hybridizable to NTP genes or transcripts, or antibodies specific for an NTP. By "neurological disease" is meant Alzheimer's Disease (AD), or other neurodegenerative disorders with the Alzheimer's type pathogenic changes (for example,

Parkinson's disease with AD-type neurodegeneration), as well as neuroectodermal tumors, malignant astrocytomas, and glioblastomas. By "human subject" is meant any human being or any developmental form thereof, such as a human embryo or fetus, prior to birth. The diagnostic methods of the present invention do not require invasive removal of neural tissue.

The present invention additionally pertains to assays, both nucleic acid hybridization assays and immunoassays, for detecting the presence of NTP in cells or in the biological fluids of a human subject using light or electron microscopic histology, imaging, radioactive or enzyme based assays, and the like.

A. Nucleic Acid Hybridization Assays

In testing a tissue sample for an NTP using a nucleic acid hybridization assay, RNA can be isolated from tissue by sectioning on a cryostat and lysing the sections with a detergent such as SDS and a chelating agent such as EDTA, optionally with overnight digestion with proteinase K (50 μg/ml). Such tissue is obtained by autopsy and biopsy. A preferred quantity of tissue is in the range of 1-10 milligrams. Protein is removed by phenol and chloroform extractions, and nucleic acids are precipitated with ethanol. RNA is isolated by chromatography on an oligo dT column and then eluted therefrom. Further fractionation can also be carried out, according to methods well known to those of ordinary skill in the art.

A number of techniques for molecular hybridization are used for the detection of DNA or RNA sequences in tissues; each has certain advantages and disadvantages. When large amounts of tissue are available, analysis of hybridization kinetics provides the opportunity to accurately quantitate the amount of DNA or RNA present, as well as to distinguish sequences that are closely related but not identical to the probe, and determine the percent homology.

Reactions are run under conditions of hybridization (Tm-25°C) in which the rate of reassociation of the probe is optimal (Wetmur *et al., J. Mol. Biol. 31*:349-370 (1968)). The kinetics of the reaction are second- order when the sequences in the tissue are identical to those of the probe; however, the reaction exhibits complex kinetics when probe sequences have partial homology to those in the tissue (Sharp *et al., J. Mol. Biol. 86*:709-726 (1974)).

The ratio of probe to cell RNA is determined by the sensitivity desired. To detect one transcript per cell would require about 100 pg of probe per μg of total cellular DNA or RNA. The nucleic acids are mixed, denatured, brought to the appropriate salt concentration and temperature, and allowed to hybridize for various periods of time. The rate of reassociation can be determined by quantitating the amount of probe hybridized either by hydroxy apatite chromatography (Britten *et al., Science 161*:529-540 (1968)) or S1 nuclease digestion (Sutton, *Biochim. Biophys. Acta 240*:522-531 (1971)).

A more flexible method of hybridization is the northern blot technique. This technique offers variability in the stringency of the hybridization reaction, as well as determination of the state of the retroviral sequences in the specimen under analysis. Northern analysis can be performed as described herein.

A major consideration associated with hybridization analysis of DNA or RNA sequences is the degree of relatedness the probe has with the sequences present in the specimen under study. This is important with the blotting technique, since a moderate degree of sequence homology under nonstringent conditions of hybridization can yield a strong signal even though the probe and sequences in the sample represent non-homologous genes.

The particular hybridization technique is not essential to the invention, any technique commonly used in the art being within the scope of the present invention. Typical probe technology is described in United States Patent 4,358,535 to Falkow *et al.*, incorporated by reference herein. For example, hybridization can be carried out in a solution containing 6 x SSC (10 x SSC:

1.5 M sodium chloride, 0.15 M sodium citrate, pH 7.0), 5 x Denhardt's (1 x Denhardt's: 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone, 0.02% Ficoll 400), 10 mM EDTA, 0.5% SDS and about 10^7 cpm of nick-translated DNA for 16 hours at 65°C.

The labeled probes, as described above, provide a general diagnostic method for detection of an NTP in tissue. The method is reasonably rapid, has a simple protocol, has reagents which can be standardized and provided as commercial kits, and allows for rapid screening of large numbers of samples.

In one method for carrying out the procedure, a clinical isolate containing RNA transcripts is fixed to a support. The affixed nucleic acid is contacted with a labeled polynucleotide having a base sequence complementary or homologous to the coding strand of the NTP gene.

The hybridization assays of the present invention are particularly well suited for preparation and commercialization in kit form, the kit comprising a carrier means compartmentalized to receive one or more container means (vial, test tube, etc.) in close confinement, each of said container means comprising one of the separate elements to be used in hybridization assay.

For example, there may be a container means containing NTP cDNA molecules suitable for labeling by "nick translation" (*see*, for example, *Sambrook et al., supra,* for standard methodology), or labeled NTP cDNA or RNA molecules. Further container means may contain standard solutions for nick translation of NTP cDNA comprising DNA polymerase I/DNase I and unlabeled deoxyribonucleotides (i.e., dCTP, dTTP, dGTP, and dATP).

The presence of NTP RNA is determined by the variation in the appearance and/or quantity of probe-related RNA in tested tissue.

The DNA probes of this invention can also be used for differential diagnosis of hereditary or familial AD and non-hereditary or sporadic AD. The familial form of AD often occurs at an earlier age and is associated with Down's syndrome in the family. Thus, a genetic test for familial AD allows for genetic counseling of families. While much effort has been directed

toward characterizing a genetic marker for familial AD (Gusella, *FASEB J 3*:2036-2041 (1989); Hooper, *J NIH Res. 4*:48-54 (1992)), genetic linkage analysis only identifies a genetic marker sequence without providing the knowledge of the function of the genomic sequence. In contrast, the cDNA probes described herein and obtained from individuals with sporatic AD encode a known protein of known function which is over-expressed in brain tissue of patients with AD.

Most cases of the AD disorder appear to be the sporadic form, although there are well-documented familial cases (Gusella, *supra*; *Harrison's Principles of Internal Medicine*, Braunwald *et al.*, Eds., Eleventh Edition, McGraw-Hill Book Company, New York, pp. 2012-2013 (1987)). A patient with familial AD, unlike a patient with sporadic AD, inherited the predisposing mutation through the germ cells. Some of the familial cases have been shown to follow an autosomal dominant pattern of inheritance (*Id.*). Thus, the DNA of a patient with familial AD will contain the inherited genetic alteration which is absent from the DNA of a patient with sporadic AD.

A method of differentiating between sporadic and familial AD in a human subject involves obtaining a biological sample from the human subject who is suspected of having Alzheimer's Disease. Then, DNA is purified from the biological sample. Finally, the DNA is contacted with a NTP DNA probe under conditions of hybridization. Familial AD is indicated by the detection of a hybrid of the probe and the DNA, whereas sporadic AD is indicated by the absence of detection of hybridization.

For example, the biological sample can be a blood sample which is subjected to differential centrifugation to enrich for white blood cells within three days of collection (Park, "PCR in the Diagnosis of Retinoblastoma," in *PCR Protocols*, Innis *et al.*, Eds., Academic Press, Inc., New York, pp. 407-415 (1990)). The DNA sample can be prepared using the sodium N-lauroylsarcosine-Proteinase K, phenol, and RNase method (Sambrook *et al.*, *supra*). DNA analysis can be performed by digesting the DNA sample, preferably 5 micrograms, with a restriction endonuclease (such as *Hind*III).

Digested DNA is then fractionated using agarose gel electrophoresis, preferably, a 1% horizontal agarose gel, for 18 hours in a buffer preferably containing 89 mM Tris-Hcl (pH 8), 89 mM sodium borate and 2 mM EDTA (Gusella *et al., Nature 306*:234-238 (1983)). Southern analysis can be performed using conventional techniques (Sambrook *et al., supra*), and the labelled AD cDNA probes can be hybridized under conditions described above. The preferred DNA probes for this differential diagnosis method include 1-9a, AD3-4, AD4-4 and G2-2 PstI.

B. Immunoassays

Antibodies directed against an NTP can be used, as taught by the present invention, to detect and diagnose AD. Various histological staining methods, including immunohistochemical staining methods, may also be used effectively according to the teaching of the invention. Silver stain is but one method of visualizing NTP. Other staining methods useful in the present invention will be obvious to the artisan, the determination of which would not involve undue experimentation (*see generally*, for example, *A Textbook of Histology*, Eds. Bloom and Fawcett, W.B. Saunders Co., Philadelphia (1964)).

One screening method for determining whether a given compound is an NTP functional derivative comprises, for example, immunoassays employing radioimmunoassay (RIA) or enzyme-linked immunosorbant assay (ELISA) methodologies, based on the production of specific antibodies (monoclonal or polyclonal) to an NTP. For these assays, biological samples are obtained by venepuncture (blood), spinal tap (cerebral spinal fluid (CSF)), urine and other body secretions such as sweat and tears. For example, in one form of RIA, the substance under test is mixed with diluted antiserum in the presence of radiolabeled antigen. In this method, the concentration of the test substance will be inversely proportional to the amount of labeled antigen bound to the specific antibody and directly related to the amount of free

labeled antigen. Other suitable screening methods will be readily apparent to those of skill in the art.

The present invention also relates to methods of detecting an NTP or functional derivatives in a sample or subject. For example, antibodies specific for an NTP, or a functional derivative, may be detectably labeled with any appropriate marker, for example, a radioisotope, an enzyme, a fluorescent label, a paramagnetic label, or a free radical.

Alternatively, antibodies specific for an NTP, or a functional derivative, may be detectably labeled with DNA by the technique of immuno-polymerase chain reaction (Sano *et al., Science 258*: 120-122 (1992)). The polymerase chain reaction (PCR) procedure amplifies specific nucleic acid sequences through a series of manipulations including denaturation, annealing of oligonucleotide primers, and extension of the primers with DNA polymerase (*see*, for example, Mullis *et al.*, U.S. Patent No. 4,683,202; Mullis *et al.*, U.S. Patent No. 4,683,195; Loh *et al., Science 243*:217 (1988)). The steps can be repeated many times, resulting in a large amplification of the number of copies of the original specific sequence. As little as a single copy of a DNA sequence can be amplified to produce hundreds of nanograms of product (Li *et al., Nature 335*:414 (1988)). Other known nucleic acid amplification procedures include transcription-based amplification systems (Kwoh *et al., Proc. Natl. Acad. Sci. USA 86*:1173 (1989); Gingeras *et al.*, WO 88/10315), and the "ligase chain reaction" in which two (or more) oligonucleotides are ligated in the presence of a nucleic acid target having the sequence of the resulting "di-oligonucleotide" thereby amplifying the di-oligonucleotide (Wu *et al., Genomics 4*:560 (1989); Backman *et al.*, EP 320,308; Wallace, EP 336,731; Orgel, WO 89/09835).

For example, the immuno-PCR assay can be carried out by immobilizing various amounts of the test material on the surface of microtiter wells (*see* Sanzo *et al., supra*, page 122, footnote 7). The wells are subsequently incubated with an NTP monoclonal antibody, washed, and then incubated with biotinylated NTP DNA molecules which have been conjugated

to streptavidin-protein chimera (*Id.*). This chimera binds biotin (via the streptavidin moiety) and the Fc portion of an immunoglobulin G molecule (via the protein A moiety) (*Id.*, at 120; Sanzo *et al.*, *Bio/Technology* 9:1378 (1991)). The wells are then washed to remove unbound conjugates. Any NTP present in the test material will be bound by the NTP monoclonal antibody, which in turn, is bound by the protein A moiety of the biotinylated NTP DNA - streptavidin-protein A conjugate. Then, the NTP DNA sequences are amplified using PCR. Briefly, the microtiter wells are incubated with deoxyribonucleoside triphosphates, NTP oligonucleotide primers, and Taq DNA polymerase (*see* Sanzo *et al.*, *supra*, page 122, footnote 11). An automated thermal cycler (such as the PTC-100-96 Thermal Cycler, MJ Research, Inc.) can be used to perform PCR under standard conditions (*Id.*). The PCR products are then analyzed by agarose gel electrophoresis after staining with ethidium bromide.

Methods of making and detecting such detectably labeled antibodies or their functional derivatives are well known to those of ordinary skill in the art, and are described in more detail below. Standard reference works setting forth the general principles of immunology include the work of Klein (*Immunology: The Science of Self-Nonself Discrimination*, John Wiley & Sons, New York (1982)); Kennett *et al.* (*Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, New York (1980)); Campbell ("Monoclonal Antibody Technology," In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Volume 13 (Burdon, R., *et al.*, eds.), Elsevier, Amsterdam (1984)); and Eisen (In: *Microbiology*, 3rd Ed. (Davis, *et al.*, Harper & Row, Philadelphia (1980)).

The term "antibody" refers both to monoclonal antibodies which are a substantially homogeneous population and to polyclonal antibodies which are heterogeneous populations. Polyclonal antibodies are derived from the sera of animals immunized with an antigen. Monoclonal antibodies (mAbs) to specific antigens may be btained by methods known to those skilled in the art. *See*, for example, Kohler and Milstein, *Nature* 256:495-497 (1975) and

U.S. Patent No. 4,376,110. Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof.

The monoclonal antibodies, particularly mAbs Th7, Th9, and Th10 used in the present invention, may be prepared as previously described (Gross *et al., J. Clin. Invest. 76*:2115-2126 (1985); Ozturk *et al., Proc. Natl. Acad. Sci. USA 86*:419-423 (1989); de la Monte *et. al., J. Clin. Invest. 86*:1004-1013 (1990); de la Monte *et. al., J. Neurol. Sci. 113*:152-164 (1992); de la Monte *et al., Ann. Neurol. 32*:733-742 (1992)). The Th monoclonal antibodies were generated against the purified pancreatic form of thread protein (*Id.*). NTP-specific polyclonal and monoclonal antibodies can also be generated against a substantially pure NTP isolated from recombinant hosts (for example, *see* Carroll *et al.*, "Production and Purification of Polyclonal Antibodies to the Foreign Segment of ß-Galactosidase Fusion Proteins," in *DNA Cloning: A Practical Approach*, Volume III, IRL Press, Washington, D.C., pp. 89-111 (1987); Mole *et al.*, "Production of Monoclonal Antibodies Against Fusion Proteins Produced in *Escherichia coli*," in *DNA Cloning: A Practical Approach*, Volume III, IRL Press, Washington, D.C., pp. 113-1139 (1987)). Alternatively, NTP-specific polyclonal and monoclonal antibodies can be generated against a substantially pure NTP isolated from biological material such as brain tissue and cell lines, by using well known techniques.

For example, monoclonal antibodies specific for the various NTP molecules of approximately, 8, 14, 17, 21, 26 kDa and 42 kDa molecular weights may be prepared from recombinant-derived proteins, which are expressed, isolated and purified from the cDNA (i.e., 1-9a), genomic clones (G2-2 *PstI*) and AD-NTP 3-4 cDNA clones. These NTP molecules are derived from the above cDNA's and genomic clones, inserted and produced in suitable expression vectors (*see* Figures 2A and 2B). Since there are regi ns of 60-70% homology in the 5' ends of the 1-9a NTP cDNA and PTP, one can obtain monoclonal antibodies that bind specifically to the NTP recombinant proteins and not to the pancreatic form by performing routine differential screening (*see*, for example, de la Monte *et al., J. Clin. Invest.*

86: 1004-1013 (1990)). Although there will be monoclonal antibodies that bind to both NTP and PTP, it will be possible to generate NTP-specific monoclonal antibodies because there is a substantial sequence divergence between NTP molecules of various forms (e.g., 8, 14, 17, 21, 26 and 42 kDa) and because an epitope may be defined by as few as 6-8 amino acids.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and $F(ab')_2$, which are capable of binding antigen. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl *et al., J. Nucl. Med. 24*:316-325 (1983)).

It will be appreciated that Fab and $F(ab')_2$ and other fragments of the antibodies useful in the present invention may be used for the detection and quantitation of an NTP according to the methods disclosed herein in order to detect and diagnose AD in the same manner as an intact antibody. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments).

An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. The term "epitope" is meant to refer to that portion of any molecule capable of being bound by an antibody which can also be recognized by that antibody. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics.

An "antigen" is a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen may have one, or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its

corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The antibodies, or fragments of antibodies, useful in the present invention may be used to quantitatively or qualitatively detect the presence of cells which contain the NTP antigens. Thus, the antibodies (or fragments thereof) useful in the present invention may be employed histologically to detect or visualize the presence of an NTP.

Such an assay for an NTP typically comprises incubating a biological sample from said subject suspected of having such a condition in the presence of a detectably labeled binding molecule (e.g., antibody) capable of identifying an NTP, and detecting said binding molecule which is bound in a sample.

Thus, in this aspect of the invention, a biological sample may be treated with nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled NTP-specific antibody. The solid phase support may then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on said solid support may then be detected by conventional means.

By "solid phase support" is intended any support capable of binding antigen or antibodies. Well-known supports, or carriers, include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will note many other suitable carriers for binding

monoclonal antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

One embodiment for carrying out the diagnostic assay of the present invention on a biological sample containing an NTP, comprises:

(a) contacting a detectably labeled NTP-specific antibody with a solid support to effect immobilization of said NTP-specific antibody or a fragment thereof;

(b) contacting a sample suspected of containing an NTP with said solid support;

(c) incubating said detectably labeled NTP-specific antibody with said support for a time sufficient to allow the immobilized NTP-specific antibody to bind to the NTP;

(d) separating the solid phase support from the incubation mixture obtained in step (c); and

(e) detecting the bound label and thereby detecting and quantifying NTP.

Alternatively, labeled NTP-specific antibody/NTP complexes in a sample may be separated from a reaction mixture by contacting the complex with an immobilized antibody or protein which is specific for an immunoglobulin, e.g., *Staphylococcus* protein A, *Staphylococcus* protein G, anti-IgM or anti-IgG antibodies. Such anti-immunoglobulin antibodies may be polyclonal, but are preferably monoclonal. The solid support may then be washed with a suitable buffer to give an immobilized NTP/labeled NTP-specific antibody complex. The label may then be detected to give a measure of an NTP.

This aspect of the invention relates to a method for detecting an NTP or a fragment thereof in a sample comprising:

(a) contacting a sample suspected of containing an NTP with an NTP-specific antibody or fragment thereof which binds to NTP; and

(b) detecting whether a complex is formed.

The invention also relates to a method of detecting an NTP in a sample, further comprising:

(c) contacting the mixture obtained in step (a) with an Fc binding molecule, such as an antibody, *Staphylococcus* protein A, or *Staphylococcus* protein G, which is immobilized on a solid phase support and is specific for the NTP-specific antibody to give a NTP/NTP-specific antibody immobilized antibody complex;

(d) washing the solid phase support obtained in step (c) to remove unbound NTP/NTP-specific antibody complex;

(e) and detecting the label bound to said solid support.

Of course, the specific concentrations of detectably labeled antibody and NTP, the temperature and time of incubation, as well as other assay conditions may be varied, depending on various factors including the concentration of an NTP in the sample, the nature of the sample, and the like. The binding activity of a given lot of anti-NTP antibody may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

Other such steps as washing, stirring, shaking, filtering and the like may be added to the assays as is customary or necessary for the particular situation.

One of the ways in which the NTP-specific antibody can be detectably labeled is by linking the same to an enzyme. This enzyme, in turn, when later exposed to its substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used to detectably label the NTP-specific antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, α-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase,

asparaginase, glucose oxidase, ß-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

Detection may be accomplished using any of a variety of immunoassays. For example, by radioactively labeling the NTP-specific antibodies or antibody fragments, it is possible to detect NTP through the use of radioimmune assays. A good description of a radioimmune assay may be found in *Laboratory Techniques and Biochemistry in Molecular Biology*, by Work, *et al.*, North Holland Publishing Company, NY (1978), with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, incorporated by reference herein.

The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography. Isotopes which are particularly useful for the purpose of the present invention are: ^{3}H, ^{125}I, ^{131}I, ^{35}S, ^{14}C, and preferably ^{125}I.

It is also possible to label the NTP-specific antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labelling compounds are fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, *o*-phthaldehyde and fluorescamine.

The NTP-specific antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the NTP-specific antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The NTP-specific antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged NTP-specific antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol,

isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

The NTP-specific antibody may also be labeled with biotin and then reacted with avidin. A biotin-labeled DNA fragment will be linked to the NTP-biotinylated monoclonal antibody by an avidin bridge. NTP molecules are then detected by polymerase chain reaction (PCR) amplification of the DNA fragment with specific primers (Sano *et al., Science 258*: 120-122 (1992)).

Likewise, a bioluminescent compound may be used to label the NTP-specific antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Detection of the NTP-specific antibody may be accomplished by a scintillation counter, for example, if the detectable label is a radioactive gamma emitter, or by a fluorometer, for example, if the label is a fluorescent material. In the case of an enzyme label, the detection can be accomplished by colorimetric methods which employ a substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

The detection of foci of such detectably labeled antibodies is indicative of a disease or dysfunctional state as previously described. For the purposes of the present invention, the NTP which is detected by this assay may be present in a biological sample. Any sample containing an NTP can be used. However, one of the benefits of the present diagnostic invention is that invasive tissue removal may be avoided. Therefore, preferably, the sample is a biological solution such as, for example, cerebrospinal fluid, amniotic fluid, blood, serum, urine and the like. However, the invention is not limited to assays using only these samples, it being possible for one of ordinary skill

in the art to determine suitable conditions which allow the use of other samples.

For example, the three-site monoclonal antibody-based immunoradiometric assays (M-IRMA) may be used to measure NTP levels in a biological fluid, such as CSF. It is possible to obtain, by spinal tap, on a routine basis, CSF from individuals suspected of having AD. Thus, the diagnosis of AD can be established by a simple, non-invasive immunoassay which reveals NTP levels greatly increased over normal levels.

In one embodiment, as described above, this examination for AD is accomplished by removing samples of biological fluid and incubating such samples in the presence of detectably labeled antibodies (or antibody fragments). In a preferred embodiment, this technique is accomplished in a non-invasive manner through the use of magnetic imaging, fluorography, etc.

Preferably, the detection of cells which express an NTP may be accomplished by *in vivo* imaging techniques, in which the labeled antibodies (or fragments thereof) are provided to a subject, and the presence of the NTP is detected without the prior removal of any tissue sample. Such *in vivo* detection procedures have the advantage of being less invasive than other detection methods, and are, moreover, capable of detecting the presence of NTP in tissue which cannot be easily removed from the patient, such as brain tissue.

Using *in vivo* imaging techniques, it will be possible to differentiate between AD and a brain tumor because NTP will be detected throughout the brain in an AD patient, while NTP will be localized in discrete deposits in the case of brain tumors. For example, in brains of AD patients, NTP will be found in the temporal, parietal and frontal cortices as well as the amygdala and hippocampus. Favored cites for astrocytomas include the cerebrum, cerebellum, thalamus, optic chiasma, and pons (*Harrison's Principles of Internal Medicine*, Petersdorf *et al.*, Eds., Tenth Edition, McGraw-Hill Book Company, New York, p.2076 (1983)), and glioblastoma multiforme is predominantly cerebral in location (*Id.* at 2075).

There are many different *in vivo* labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include radioactive isotopes and paramagnetic isotopes. Those of ordinary skill in the art will know of other suitable labels for binding to the antibodies used in the invention, or will be able to ascertain such, using routine experimentation. Furthermore, the binding of these labels to the antibodies can be done using standard techniques common to those of ordinary skill in the art.

An important factor in selecting a radionuclide for *in vivo* diagnosis is that the half-life of a radionuclide be long enough so that it is still detectable at the time of maximum uptake by the target, but short enough so that deleterious radiation upon the host is minimized. Ideally, a radionuclide used for *in vivo* imaging will lack a particulate emission, but produce a large number of photons in the 140-200 keV range, which maybe readily detected by conventional gamma cameras.

For *in vivo* diagnosis radionuclides may be bound to antibody either directly or indirectly by using an intermediary functional group. Intermediary functional groups which are often used in binding radioisotopes which exist as metallic ions to immunoglobulins are DTPA and EDTA. Typical examples of ions which can be bound to immunoglobulins are ^{99m}Tc, ^{123}I, ^{111}In, ^{131}I, ^{97}Ru, ^{67}Cu, ^{67}Ga, ^{125}I, ^{68}Ga, ^{72}As, ^{89}Zr, and ^{201}Tl.

For diagnostic *in vivo* imaging, the type of detection instrument available is a major factor in selecting a given radionuclide. The radionuclide chosen must have a type of decay which is detectable for a given type of instrument. In general, any conventional method for visualizing diagnostic imaging can be utilized in accordance with this invention. For example, PET, gamma, beta, and MRI detectors can be used to visualize diagnostic imagining.

The antibodies useful in the invention can also be labeled with paramagnetic isotopes for purposes of *in vivo* diagnosis. Elements which are

particularly useful, as in Magnetic Resonance Imaging (MRI), include ^{157}Gd, ^{55}Mn, ^{162}Dy, and ^{56}Fe.

The antibodies (or fragments thereof) useful in the present invention are also particularly suited for use in *in vitro* immunoassays to detect the presence of an NTP in body tissue, fluids (such as CSF), or cellular extracts. In such immunoassays, the antibodies (or antibody fragments) may be utilized in liquid phase or, preferably, bound to a solid-phase carrier, as described above.

Those of ordinary skill in the art will know of other suitable labels which may be employed in accordance with the present invention. The binding of these labels to antibodies or fragments thereof can be accomplished using standard techniques commonly known to those of ordinary skill in the art. Typical techniques are described by Kennedy, *et al.* (*Clin. Chim. Acta 70*:1-31 (1976)) and Schurs, *et al.* (*Clin. Chim. Acta 81*:1-40 (1977)). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

In situ detection may be accomplished by removing a histological specimen from a patient, and providing the combination of labeled antibodies of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of an NTP, but also the distribution of an NTP on the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

The binding molecules of the present invention may be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In a typical immunometric assay, a quantity of unlabeled

antibody (or fragment of antibody) is bound to a solid support that is insoluble in the fluid being tested (i.e., CSF) and a quantity of detectably labeled soluble antibody is added to permit detection and/or quantitation of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

Typical, and preferred, immunometric assays include "forward" assays in which the antibody bound to the solid phase is first contacted with the sample being tested to extract the antigen from the sample by formation of a binary solid phase antibody-antigen complex. After a suitable incubation period, the solid support is washed to remove the residue of the fluid sample, including unreacted antigen, if any, and then contacted with the solution containing an unknown quantity of labeled antibody (which functions as a "reporter molecule"). After a second incubation period to permit the labeled antibody to complex with the antigen bound to the solid support through the unlabeled antibody, the solid support is washed a second time to remove the unreacted labeled antibody. This type of forward sandwich assay may be a simple "yes/no" assay to determine whether antigen is present or may be made quantitative by comparing the measure of labeled antibody with that obtained for a standard sample containing known quantities of antigen. Such "two-site" or "sandwich" assays are described by Wide at pages 199-206 of *Radioimmune Assay Method*, edited by Kirkham and Hunter, E. & S. Livingstone, Edinburgh, 1970.

In another type of "sandwich" assay, which may also be useful with the antigens of the present invention, the so-called "simultaneous" and "reverse" assays are used. A simultaneous assay involves a single incubation step as the antibody bound to the solid support and labeled antibody are both added to the sample being tested at the same time. After the incubation is completed, the solid support is washed to remove the residue of fluid sample and uncomplexed labeled antibody. The presence of labeled antibody associated with the solid support is then determined as it would be in a conventional "forward" sandwich assay.

In the "reverse" assay, stepwise addition first of a solution of labeled antibody to the fluid sample followed by the addition of unlabeled antibody bound to a solid support after a suitable incubation period is utilized. After a second incubation, the solid phase is washed in conventional fashion to free it of the residue of the sample being tested and the solution of unreacted labeled antibody. The determination of labeled antibody associated with a solid support is then determined as in the "simultaneous" and "forward" assays.

The above-described *in vitro* or *in vivo* detection methods may be used in the detection and diagnosis of AD without the necessity of removing tissue. Such detection methods may be used to assist in the determination of the stage of neurological deterioration in AD by evaluating and comparing the concentration of an NTP in the biological sample.

As used herein, an effective amount of a diagnostic reagent (such as an antibody or antibody fragment) is one capable of achieving the desired diagnostic discrimination and will vary depending on such factors as age, condition, sex, the extent of disease of the subject, counterindications, if any, and other variables to be adjusted by the physician. The amount of such materials which are typically used in a diagnostic test are generally between 0.1 to 5 mg, and preferably between 0.1 to 0.5 mg.

The assay of the present invention is also ideally suited for the preparation of a kit. Such a kit may comprise a carrier means being compartmentalized to receive in close confinement therewith one or more container means such as vials, tubes and the like, each of said container means comprising the separate elements of the immunoassay.

For example, there may be a container means containing a first antibody immobilized on a solid phase support, and a further container means containing a second detectably labeled antibody in solution. Further container means may contain standard solutions comprising serial dilutions of the NTP to be detected. The standard solutions of an NTP may be used to prepare a standard curve with the concentration of NTP plotted on the abscissa and the

detection signal on the ordinate. The results obtained from a sample containing an NTP may be interpolated from such a plot to give the concentration of the NTP.

IV. Isolation of NTP

The NTP proteins or fragments of this invention may be obtained by expression from recombinant DNA as described above. Alternatively, an NTP may be purified from biological material.

For purposes of the present invention, one method of purification which is illustrative, without being limiting, consists of the following steps.

A first step in the purification of an NTP includes extraction of the NTP fraction from a biological sample, such as brain tissue or CSF, in buffers, with or without solubilizing agents such as urea, formic acid, detergent, or thiocyanate.

A second step includes subjecting the solubilized material to ion-exchange chromatography on Mono-Q or Mono-S columns (Pharmacia LKB Biotechnology, Inc; Piscataway, NJ). Similarly, the solubilized material may be separated by any other process wherein molecules can be separated according to charge density, charge distribution and molecular size, for example. Elution of the NTP from the ion-exchange resin are monitored by an immunoassay, such as M-IRMA, on each fraction. Immunoreactive peaks would are then dialyzed, lyophilized, and subjected to molecular sieve, or gel chromatography.

Molecular sieve or gel chromatography is a type of partition chromatography in which separation is based on molecular size. Dextran, polyacrylamide, and agarose gels are commonly used for this type of separation. One useful gel for the present invention is Sepharose 12 (Pharmacia LKB Biotechnology, Inc.). However, other methods, known to those of skill in the art may be used to effectively separate molecules based on size.

A fourth step in a purification protocol for an NTP includes analyzing the immunoreactive peaks by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), a further gel chromatographic purification step, and staining, such as, for example, silver staining.

A fifth step in a purification method includes subjecting the NTP obtained after SDS-PAGE to affinity chromatography, or any other procedure based upon affinity between a substance to be isolated and a molecule to which it can specifically bind. For further purification of an NTP, affinity chromatography on Sepharose conjugated to anti-NTP mAbs (such as Th9, or specific mABs generated against substantially pure NTP) can be used. Alternative methods, such as reverse-phase HPLC, or any other method characterized by rapid separation with good peak resolution are useful.

Another method to purify an NTP is to use concentrated CSF obtained from patients with AD. For this procedure, 30-40 milliliters are concentrated by lyophilization or Amicon filtration or the like, and subjected to two dimensional gel electrophoresis. Proteins are separated in one direction by charge in a pH gradient and then, subjected to molecular sieve chromatography in the other direction by polyacrylamide gel electrophoresis. NTP-immunoreactive proteins are identified as spots by the Th monoclonal antibodies (for example, Th 9) using Western blot anaylsis. The gel is cut and NTP proteins are eluted from the gel. NTP purified in this manner can be sequenced or used to make new monoclonal antibodies.

It will be appreciated that other purification steps may be substituted for the preferred method described above. Those of skill in the art will be able to devise alternate purification schemes without undue experimentation.

V. Gene Therapy Using Antisense Oligonucleotides and Ribozymes

Antisense oligonucleotides have been described as naturally occurring biological inhibitors of gene expression in both prokaryotes (Mizuno *et al.*, *Proc. Natl. Acad. Sci. USA 81*:1966-1970 (1984)) and eukaryotes (Heywood,

Nucleic Acids Res. 14:6771-6772 (1986)), and these sequences presumably function by hybridizing to complementary mRNA sequences, resulting in hybridization arrest of translation (Paterson, *et al., Proc. Natl. Acad. Sci. USA, 74*:4370-4374 (1987)).

Antisense oligonucleotides are short synthetic DNA or RNA nucleotide molecules formulated to be complementary to a specific gene or RNA message. Through the binding of these oligomers to a target DNA or mRNA sequence, transcription or translation of the gene can be selectively blocked and the disease process generated by that gene can be halted (*see*, for example, Jack Cohen, *Oligodeoxynucleotides, Antisense Inhibitors of Gene Expression*, CRC Press (1989)). The cytoplasmic location of mRNA provides a target considered to be readily accessible to antisense oligodeoxynucleotides entering the cell; hence much of the work in the field has focused on RNA as a target. Currently, the use of antisense oligodeoxynucleotides provides a useful tool for exploring regulation of gene expression *in vitro* and in tissue culture (Rothenberg, *et al., J. Natl. Cancer Inst. 81*:1539-1544 (1989)).

Antisense therapy is the administration of exogenous oligonucleotides which bind to a target polynucleotide located within the cells. For example, antisense oligonucleotides may be administered systemically for anticancer therapy (Smith, International Application Publication No. WO 90/09180). As described herein, NTP-related proteins are produced by neuroectodermal tumor cells, malignant astrocytoma cells, glioblastoma cells, and in relatively high concentrations (i.e, relative to controls) in brain tissue of AD patients. Thus, NTP antisense oligonucleotides of the present invention may be active in treatment against AD, as well as neuroectodermal tumors, malignant astrocytomas, and glioblastomas.

The NTP antisense oligonucleotides of the present invention include derivatives such as S-oligonucleotides (phosphorothioate derivatives or S-oligos, *see*, Jack Cohen, *supra*). S-oligos (nucleoside phosphorothioates) are isoelectronic analogs of an olig nucleotide (O-oligo) in which a nonbridging oxygen atom of the phosphate group is replaced by a sulfur atom. The S-

oligos of the present invention may be prepared by treatment of the corresponding O-oligos with 3*H*-1,2-benzodithiol-3-one-1,1-dioxide which is a sulfur transfer reagent. *See* Iyer *et al., J. Org. Chem. 55*:4693-4698 (1990); and Iyer *et al., J. Am. Chem. Soc. 112*:1253-1254 (1990), the disclosures of which are fully incorporated by reference herein.

As described herein, sequence anaylsis of an NTP cDNA clone shows that NTP contains sequences which are nonhomologous to PTP DNA sequences (*see* Figure 9). Thus, the NTP antisense oligonucleotides of the present invention may be RNA or DNA which is complementary to and stably hybridizes with such sequences which are specific for an NTP. Use of an oligonucleotide complementary to this region allows for the selective hybridization to NTP mRNA and not to mRNA specifying PTP. Preferably, the NTP antisense oligonucleotides of the present invention are a 15 to 30-mer fragment of the antisense DNA molecule coding for the nonhomologous sequences of the AD 3-4 cDNA, such as:

1. 5'-CCGATTCCAACAGACCATCAT-3' [SEQ ID NO: 1];
2. 5'-CCAACAGACCATCATTCCACC-3' [SEQ ID NO: 2]; and
3. 5'-CCAAACCGATTCCAACAGACC-3' [SEQ ID NO: 3].

Preferred antisense oligonucleotides bind to the 5'-end of the AD10-7 mRNA. Such antisense oligonucleotides may be used to down regulate or inhibit expression of the NTP gene. Examples of such antisense oligonucleotides (30-mers) include:

1. 5'-CCTGGGCAACAAGAGCGAAAACTCCATCTC-3' [SEQ ID NO: 4];
2. 5'-ATCGCTTGAACCCGGGAGGCGGAGGTTGCG-3' [SEQ ID NO: 5]; and
3. 5'-GGGGAGGCTGAGGCAGGAGAATCGCTTGAA-3'[SEQ ID NO: 6].

Included as well in the present invention are pharmaceutical compositions comprising an effective amount of at least one of the NTP antisense oligonucleotides of the invention in combination with a pharma-

ceutically acceptable carrier. In one embodiment, a single NTP antisense oligonucleotide is utilized. In another embodiment, two NTP antisense oligonucleotides are utilized which are complementary to adjacent regions of the NTP genome. Administration of two NTP antisense oligonucleotides which are complementary to adjacent regions of the genome or corresponding mRNA may allow for more efficient inhibition of NTP genomic transcription or mRNA translation, resulting in more effective inhibition of NTP production.

Preferably, the NTP antisense oligonucleotide is coadministered with an agent which enhances the uptake of the antisense molecule by the cells. For example, the NTP antisense oligonucleotide may be combined with a lipophilic cationic compound which may be in the form of liposomes. The use of liposomes to introduce nucleotides into cells is taught, for example, in U.S. Patent Nos. 4,897,355 and 4,394,448, the disclosures of which are incorporated by reference in their entirety. *See also* U.S. Patent Nos. 4,235,871, 4,231,877, 4,224,179, 4,753,788, 4,673,567, 4,247,411, 4,814,270 for general methods of preparing liposomes comprising biological materials.

Alternatively, the NTP antisense oligonucleotide may be combined with a lipophilic carrier such as any one of a number of sterols including cholesterol, cholate and deoxycholic acid. A preferred sterol is cholesterol.

In addition, the NTP antisense oligonucleotide may be conjugated to a peptide that is ingested by cells. Examples of useful peptides include peptide hormones, antigens or antibodies, and peptide toxins. By choosing a peptide that is selectively taken up by the neoplastic cells, specific delivery of the antisense agent may be effected. The NTP antisense oligonucleotide may be covalently bound via the 5'OH group by formation of an activated aminoalkyl derivative. The peptide of choice may then be covalently attached to the activated NTP antisense oligonucleotide via an amino and sulfhydryl reactive hetero bifunctional reagent. The latter is bound to a cysteine residue present in the peptide. Upon exposure of cells to the NTP antisense

oligonucleotide bound to the peptide, the peptidyl antisense agent is endocytosed and the NTP antisense oligonucleotide binds to the target NTP mRNA to inhibit translation (Haralambid *et al.*, WO 8903849; Lebleu *et al.*, EP 0263740).

The NTP antisense oligonucleotides and the pharmaceutical compositions of the present invention may be administered by any means that achieve their intended purpose. For example, administration may be by parenteral, subcutaneous, intravenous, intramuscular, intra-peritoneal, or transdermal routes. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

Compositions within the scope of this invention include all compositions wherein the NTP antisense oligonucleotide is contained in an amount effective to achieve inhibition of proliferation and/or stimulate differentiation of the subject cancer cells, or alleviate AD. While individual needs vary, determination of optimal ranges of effective amounts of each component is with the skill of the art. Typically, the NTP antisense oligonucleotide may be administered to mammals, e.g. humans, at a dose of 0.005 to 1 mg/kg/day, or an equivalent amount of the pharmaceutically acceptable salt thereof, per day of the body weight of the mammal being treated.

Alternatively, antisense oligonucleotides can be prepared which are designed to interfere with transcription of the NTP gene by binding transcribed regions of duplex DNA (including introns, exons, or both) and forming triple helices (Froehler *et al.*, WO 91/06626; Toole, WO 92/10590). Preferred oligonucleotides for triple helix formation are oligonucleotides which have inverted polarities for at least two regions of the oligonucleotide (*Id.*). Such oligonucleotides comprise tandem sequences of opposite polarity such as 3'—5'-L-5'—3', or 5'—3'-L-3'—5', wherein L represents a 0-10 base oligonucleotide linkage between oligonucleotides. The inverted polarity form stabilizes single-stranded oligonucleotides to exonuclease degradation

(Froehler *et al., supra*). Preferred triple helix-forming oligonucleotides are based upon SEQ ID NOs 1-3:

1. 3'-TACTACCAGACAACCTTAGCC-5'-L-5'-CCGATTCCAACAGACCATCAT-3' [SEQ ID NO: 7];
2. 5'-CCGATTCCAACAGACCATCAT-3'-L-3'-TACTACCAGACAACCTTAGCC-5' [SEQ ID NO: 8];
3. 3'-CCACCTTACTACCAGACAACC-5'-L-5'-CCAACAGACCATCATTCCACC-3' [SEQ ID NO: 9];
4. 5'-CCAACAGACCATCATTCCACC-3'-L-3'-CCACCTTACTACCAGACAACC-5' [SEQ ID NO: 10];
5. 3'-CCAGACAACCTTAGCCAAACC-5'-L-5'-CCAAACCGATTCCAACAGACC-3' [SEQ ID NO: 11];

and

6. 5'-CCAAACCGATTCCAACAGACC-3'-L-3'-CCAGACAACCTTAGCCAAACC-5' [SEQ ID NO: 12].

Thus, triple helix-forming oligonucleotides 1 and 2 are represented as 3'[SEQ ID NO: 1]5'-L-5'[SEQ ID NO: 1]3' and 5'[SEQ ID NO: 1]3'-L-3'[SEQ ID NO: 1]5', respectively. Triple helix-forming oligonucleotides 3 and 4 are represented as 3'[SEQ ID NO: 2]5'-L-5'[SEQ ID NO: 2]3' and 5'[SEQ ID NO: 2]3'-L-3'[SEQ ID NO: 2]5', respectively. Triple helix-forming oligonucleotides 5 and 6 are represented as 3'[SEQ ID NO: 3]5'-L-5'[SEQ ID NO: 3]3' and 5'[SEQ ID NO: 3]3'-L-3'[SEQ ID NO: 3]5', respectively. Of course, similar triple helix-forming oligonucleotide may be prepared with SEQ ID NOs. 4-6, or fragments thereof.

In therapeutic application, the triple helix-forming oligonucleotides can be formulated in pharmaceutical preparations for a variety of modes of administration, including systemic or localized administration, as described above.

The antisense oligonucleotides of the present invention may be prepared according to any of the methods that are well known to those of ordinary skill in the art, as described above.

Ribozymes provide an alternative method to inhibit mRNA function. Ribozymes may be RNA enzymes, self-splicing RNAs, and self-cleaving RNAs (Cech *et al.*, *Journal of Biological Chemistry 267*:17479-17482 (1992)). It is possible to construct *de novo* ribozymes which have an endonuclease activity directed in *trans* to a certain target sequence. Since these ribozymes can act on various sequences, ribozymes can be designed for virtually any RNA substrate. Thus, ribozymes are very flexible tools for inhibiting the expression of specific genes and provide an alternative to antisense constructs.

A ribozyme against chloramphenicol acetyltransferase mRNA has been successfully constructed (Haseloff *et al.*, *Nature 334*:585-591 (1988); Uhlenbeck *et al.*, *Nature 328*:596-600 (1987)). The ribozyme contains three structural domains: 1) a highly conserved region of nucleotides which flank the cleavage site in the 5' direction; 2) the highly conserved sequences contained in naturally occurring cleavage domains of ribozymes, forming a base-paired stem; and 3) the regions which flank the cleavage site on both sides and ensure the exact arrangement of the ribozyme in relation to the cleavage site and the cohesion of the substrate and enzyme. RNA enzymes constructed according to this model have already proved suitable *in vitro* for the specific cleaving of RNA sequences (Haseloff *et al.*, *supra*).

Alternatively, hairpin ribozymes may be used in which the active site is derived from the minus strand of the satellite RNA of tobacco ring spot virus (Hampel *et al.*, *Biochemistry 28*:4929-4933 (1989)). Recently, a hairpin ribozyme was designed which cleaves human immunodeficiency virus type 1 RNA (Ojwang *et al.*, *Proc. Natl. Acad. Sci. USA 89*:10802-10806 (1992)). Other self-cleaving RNA activities are associated with hepatitis delta virus (Kuo *et al.*, *J. Virol. 62*:4429-4444 (1988)).

As discussed above, preferred targets for NTP ribozymes are the nucleotide sequences which are not homologous with PTP sequences. Preferably, the NTP ribozyme molecule of the present invention is designed based upon the chloramphenicol acetyltransferase ribozyme or hairpin ribozymes, described above. Alternatively, NTP ribozyme molecules are

designed as described by Eckstein *et al.* (International Publication No. WO 92/07065) who disclose catalytically active ribozyme constructions which have increased stability against chemical and enzymatic degradation, and thus are useful as therapeutic agents.

In an alternative approach, an external guide sequence (EGS) can be constructed for directing the endogenous ribozyme, RNase P, to intracellular NTP mRNA, which is subsequently cleaved by the cellular ribozyme (Altman *et al.*, U.S. Patent No. 5,168,053). Preferably, the NTP EGS comprises a ten to fifteen nucleotide sequence complementary to an NTP mRNA and a 3'-NCCA nucleotide sequence, wherein N is preferably a purine (*Id.*). After NTP EGS molecules are delivered to cells, as described below, the molecules bind to the targeted NTP mRNA species by forming base pairs between the NTP mRNA and the complementary NTP EGS sequences, thus promoting cleavage of NTP mRNA by RNase P at the nucleotide at the 5'side of the base-paired region (*Id.*).

Included as well in the present invention are pharmaceutical compositions comprising an effective amount of at least one NTP ribozyme or NTP EGS of the invention in combination with a pharmaceutically acceptable carrier. Preferably, the NTP ribozyme or NTP EGS is coadministered with an agent which enhances the uptake of the ribozyme or NTP EGS molecule by the cells. For example, the NTP ribozyme or NTP EGS may be combined with a lipophilic cationic compound which may be in the form of liposomes, as described above. Alternatively, the NTP ribozyme or NTP EGS may be combined with a lipophilic carrier such as any one of a number of sterols including cholesterol, cholate and deoxycholic acid. A preferred sterol is cholesterol.

The NTP ribozyme or NTP EGS, and the pharmaceutical compositions of the present invention may be administered by any means that achieve their intended purpose. For example, administration may be by parenteral, subcutaneous, intravenous, intramuscular, intra-peritoneal, or transdermal routes. The dosage administered will be dependent upon the age, health, and

weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. For example, as much as 700 milligrams of antisense oligodeoxynucleotide has been administered intravenously to a patient over a course of 10 days (i.e., 0.05 mg/kg/hour) without signs of toxicity (Sterling, "Systemic Antisense Treatment Reported," *Genetic Engineering News 12(12)*:1, 28 (1992)).

Compositions within the scope of this invention include all compositions wherein the NTP ribozyme or NTP EGS is contained in an amount which is effective to achieve inhibition of proliferation and/or stimulate differentiation of the subject cancer cells, or alleviate AD. While individual needs vary, determination of optimal ranges of effective amounts of each component is with the skill of the art.

In addition to administering the NTP antisense oligonucleotides, ribozymes, or NTP EGS as a raw chemical in solution, the therapeutic molecules may be administered as part of a pharmaceutical preparation containing suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the NTP antisense oligonucleotide, ribozyme, or NTP EGS into preparations which can be used pharmaceutically.

Suitable formulations for parenteral administration include aqueous solutions of the NTP antisense oligonucleotides, ribozymes, NTP EGS in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

Alternatively, NTP antisense RNA molecules, NTP ribozymes, and NTP EGS can be coded by DNA constructs which are administered in the

form of virions, which are preferably incapable of replicating *in vivo* (*see*, for example, Taylor, WO 92/06693). For example, such DNA constructs may be administered using herpes-based viruses (Gage *et al.*, U.S. Patent No. 5,082,670). Alternatively, NTP antisense RNA sequences, NTP ribozymes, and NTP EGS can be coded by RNA constructs which are administered in the form of virions, such as retroviruses. The preparation of retroviral vectors is well known in the art (*see*, for example, Brown *et al.*, "Retroviral Vectors," in *DNA Cloning: A Practical Approach*, Volume 3, IRL Press, Washington, D.C. (1987)).

Specificity for gene expression in the central nervous system can be conferred by using appropriate cell-specific regulatory sequences, such as cell-specific enhancers and promoters. For example, such sequences include the sequences that regulate the oligodendroglial-specific expression of JC virus, glial-specific expression of the proteolipid protein, and the glial fibrillary acidic protein genes (Gage *et al.*, *supra*). Since protein phosphorylation is critical for neuronal regulation (Kennedy, "Second Messengers and Neuronal Function," in *An Introduction to Molecular Neurobiology*, Hall, Ed., Sinauer Associates, Inc. (1992)), protein kinase promoter sequences can be used to achieve sufficient levels of NTP gene expression.

Thus, gene therapy can be used to alleviate AD by inhibiting the inappropriate expression of a particular form of NTP. Moreover, gene therapy can be used to alleviate AD by providing the appropriate expression level of a particular form of NTP. In this case, particular NTP nucleic acid sequences may be coded by DNA or RNA constructs which are administered in the form of viruses, as described above. Alternatively, "donor cells" may be modified *in vitro* using viral or retroviral vectors containing NTP sequences, or using other well known techniques of introducing foreign DNA into cells (*see*, for example, *Sambrook et al.*, *supra*). Such donor cells include fibroblast cells, neuronal cells, glial cells, and connective tissue cells (Gage *et al.*, *supra*). Following genetic manipulation, the donor cells are

grafted into the central nervous system and thus, the genetically-modified cells provide the therapeutic form of NTP (*Id.*).

Moreover, such virions may be introduced into the blood stream for delivery to the brain. This is accomplished through the osmotic disruption of the blood brain barrier prior to administration of the virions (*see*, for example, Neuwelt, United States Patent No. 4,866,042). The blood brain barrier may be disrupted by administration of a pharmaceutically effective, nontoxic hypertonic solution, such as mannitol, arabinose, or glycerol (*Id.*).

The following clones in *E. coli* were deposited according to the Budapest Treaty with the American Type Culture Collection (12301 Parklawn Drive, Rockville, Maryland, 20852): G2-2 PstI-DH5 (ATCC No. 69257); G5d-PstI-DH5 (ATCC No. 69258); 1-9a-LX-1 blue (ATCC No. 69259); AD3-4-DH1 (ATCC No. 69260); HB4-XL-blue (ATCC No. 69261); AD10-7-DH1 (ATCC No. 69262); AD2-2-DH1- (ATCC No. 69263); G5d-1PstI-EcoRI-DH5 (ATCC No. 69264); and G2-2PstI-EcoRI-DH5 (ATCC No. 69265).

Having now generally described the invention, the same will be more readily understood through reference to the following Examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Example 1

Expression of NTP Immunoreactivity in Cell Lines

Seven cell lines of central nervous system origin were identified that express thread protein immunoreactivity using the Th9 monoclonal antibody which was generated to the pancreatic form of the protein (Gross *et al.*, *J. Clin. Invest.* *76*:2115-2126 (1985)), but cross-reacts with thread proteins present in brain tissue and cerebrospinal fluid (Ozturk *et al.*, *Proc. Natl. Acad. Sci. USA* *86*:419-423 (1989); de la Monte *et. al.*, *J. Clin. Invest.*

86:1004-1013 (1990); de la Monte *et. al., J. Neurol. Sci. 113*:152-164 (1992); de la Monte *et al., Ann. Neurol. 32*:733-742 (1992)). Among them were the following: two primitive neuroectodermal tumor (PNET) cell lines designated PNET1 and PNET2; three glioblastoma cell lines Hgl 16, Hg1 17, and C6; the A172 glial cell line; and the SH-Sy5y neuroblastoma cell line. The glioblastoma cell lines and the A172 cells were obtained from the American Type Culture Collection (ATCC). SH-Sy5y cells were obtained from Dr. Biedler at Sloan-Kettering Memorial Hospital. The PNET cell lines have been described previously (The *et al., Nature genetics 3*:62-66 (1993)), and were obtained from Dr. Rene' Bernards at the MGH Cancer Center. All cell lines were maintained in Earl's Modified Eagle Medium supplemented with 10% fetal calf serum, and without antibiotics.

To examine the cells for thread protein and other immunoreactivities, the cultures were harvested in phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO4, 1.4 mM KH_2PO_4, pH 7.3) containing 2 mM EDTA, and cytospin preparations were made using 10^5 cells per slide. The cytospin preparations were fixed immediately in 100% methanol (-20°C), air-dried, and then stored at -80°C until used. Prior to immunostaining, the slides were equilibrated to room temperature and hydrated in PBS. Nonspecific antibody binding was blocked with 3% nonimmune horse serum. Replicate cytospin preparations from the same cultures were incubated overnight at 4°C with 5 or 10 μg/ml of primary antibody. Immunoreactivity was revealed by the avidin-biotin horseradish peroxidase method using the Vectastain Elite kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's protocol, and with 3-3' diaminobenzidine (0.5 mg/ml plus 0.03% hydrogen peroxide) as the chromogen. The cells then were counterstained with hematoxylin, dehydrated in graded alcohol solutions, cleared in xylenes, and preserved under coverglass with Permount (Fisher Scientific).

Cytospin preparations of each cell line were immunostained with the thread protein monoclonal antibodies Th9, Th7, Th10, Th29, Th34, TH46,

Th67, and Th90. In addition, replicate slides were immunostained with positive (neurofilament, glial fibrillary acidic protein (GFAP), and vimentin) and negative (desmin, Hepatitis B surface antigen-5C3) control monoclonal antibodies. Except for 5C3 which was generated in the inventor's laboratory (Fujita *et al., Gastroenterology 91*:1357-1363 (1986)), the control antibodies were purchased (Boehringer-Mannheim). All serological reagents were diluted in PBS containing 1% bovine serum albumin (BSA), and all incubations except the one with primary antibody were carried out at room temperature in humidified chambers. The slides were washed in 3 changes of PBS between each step.

Both PNET1 and PNET2 cells expressed high and middle molecular weight neurofilament proteins and little or no glial fibrillary acidic protein or vimentin. The PNET1, PNET2, and SH-Sy5y cells expressed GAP-43, an abundant calmodulin-binding phosphoprotein that is highly expressed in immature neurons and in neurons undergoing regenerative cell growth (Benowitz *et al., J. Neurosci. 3*:2153-2163 (1983); DeGraan *et al., Neurosci. Lett. 61*:235-241 (1985); Kalil *et al., J. Neurosci. 6*:2563-2570 (1986)). The A172 and C6 cells expressed GFAP and vimentin. However, A172 also exhibited neurofilament immunoreactivity, raising doubt about its purely glial cell nature. None of the cell lines manifested immunoreactivity with monoclonal antibodies to desmin or to Hepatitis B surface antigen. As a negative control cell line, the Huh7 hepatocellular carcinoma cell line was similarly immunostained, and found not to exhibit any immunoreactivity with the above antibodies. However, the Huh cells were immunoreactive with monoclonal antibodies to the insulin receptor substrate protein, IRS-1 (data not shown) which was used as a positive control for this cell line (Sasaki *et al., J. Biol. Chem. 268*:1-4 (1993)).

Using the Th9 monoclonal antibody, thread protein immunoreactivity was detected in primary PNET (A), primary glioblastoma (F), PNET1 (B), and C6 cells (G), but not in hepatocellular carcinoma cell lines (Figures 1A-1J). In addition, Th9 immunoreactivity was detected in histological sections

from 8 of the 9 primary human CNS PNETs, and from all 5 of the primary human glioblastomas studied (Figures 1A-1J). Although all 5 cell lines exhibited intense immunoreactivity with the Th9 monoclonal antibody, they differed with respect to immunoreactivity for other Th monoclonal antibodies. The immunostaining reaction generated with the Th10 (C,H), Th7 (D,I), or Th46 monoclonal antibodies was either low-level (C,D) or absent (H,I,E,J) in PNET1 (C-E) and C6 (H-J). PNET2 cells exhibited only low levels of immunoreactivity with Th7 and Th29, and they manifested no immunostaining with the other Th monoclonal antibodies. A172, C6, and SH-Sy5y cells displayed little or no immunoreactivity with Th monoclonal antibodies other than Th9. Huh7 cells exhibited no immunoreactivity with any of the thread protein monoclonal antibodies employed, whereas human pancreatic tissue was immunoreactive with all of the Th antibodies, which had been generated against the purified pancreatic form of thread protein (Gross *et al., J. Clin. Invest. 76*:2115-2126 (1985)).

Example 2

Analysis of Thread Proteins by Monoclonal Antibody-Based Immunoradiometric Assay (M-IRMA)

Cultured cells were washed in PBS and recovered in PBS containing 2 mM EDTA. The cells were pelleted by centrifugation at 1000 x g for 15 min, and then resuspended in lysis buffer containing 50 mM Tris-HCl (pH 7.5), 1% Triton X-100, 2 mM EGTA, 10 mM EDTA, 100 mM NaF, 1 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 100 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml pepstatin A, and 1 μg/ml leupeptin. The supernatant fractions obtained by centrifugation of the lysates at 14,000 x g for 10 min were used for the Western blot analysis, immunoprecipitation studies, and M-IRMA. Protein concentration was determined by the Lowry colorimetric assay. The samples were stored at -40°C.

M-IRMA is a highly sensitive two- or three-site forward sandwich assay which permits quantitation of picomolar NTP in cell lysates, tissue culture medium, tissue homogenates, and body fluids (Ozturk *et al.*, *Proc. Natl. Acad. Sci. USA 86*:419-423 (1989); de la Monte *et. al., J. Clin. Invest. 86*:1004-1013 (1990); de la Monte *et. al., J. Neurol. Sci. 113*:152-164 (1992); de la Monte *et al., Ann. Neurol. 32*:733-742 (1992); Gross *et al., J. Clin. Invest. 76*:2115-2126 (1985)). In addition, when combined with SDS-PAGE, M-IRMA can be used to determine molecular size of thread proteins and related species (Ozturk *et al.*, *Proc. Natl. Acad. Sci. USA 86*:419-423 (1989); de la Monte *et. al., J. Clin. Invest. 86*:1004-1013 (1990); de la Monte *et. al., J. Neurol. Sci. 113*:152-164 (1992); de la Monte *et al., Ann. Neurol. 32*:733-742 (1992)). M-IRMA involves capturing the immunoreactive thread proteins present in biological samples using monoclonal antibodies Th7 and Th10 affixed to a solid-phase matrix, and then detecting the captured antigen with a third radiolabeled tracer monoclonal antibody (Th9) to the same protein. Briefly, 1/4" polystyrene beads (Precision Ball, Inc) were coated with one or two monoclonal antibodies to thread proteins (usually Th7 + Th10). Cell lysates or supernatant fractions of tissue homogenates (Ozturk *et al.*, *Proc. Natl. Acad. Sci. USA 86*:419-423 (1989); de la Monte *et. al., J. Clin. Invest. 86*:1004-1013 (1990); de la Monte *et. al., J. Neurol. Sci. 113*:152-164 (1992); de la Monte *et al., Ann. Neurol. 32*:733-742 (1992)) were incubated over night with the coated beads to capture thread proteins present in the samples. The beads were washed 5x in PBS, and then incubated with ^{125}-I labeled Th9 as a tracer to detect the captured thread proteins. The concentration of thread protein in the lysate or tissue homogenate was determined from a standard curve generated with known quantities of purified thread protein. This highly sensitive assay can detect as little as 10 pmol of thread protein in solution. To assay for thread proteins fractionated by SDS-PAGE, the wet gels were sliced at 2 mm intervals, and the proteins were eluted from each fraction into 0.5 ml of PBS by shaking for 24 hours at room temperature. The eluates were assayed directly for thread proteins by M-IRMA.

Corresponding with the widespread immunocytochemical staining of PNET1 cells with Th7, Th10, Th34, and Th29, thread protein immunoreactivity was readily measured in these cells by M-IRMA. In other words, with Th7, Th10, Th34, and Th29 monoclonal antibodies (MoAb) used as capture antibodies, either singularly or with two of them together, and ^{125}-I labeled Th9 was used as the tracer, similarly high levels of thread protein were measured (Figure 2). In contrast, in PNET2, C6, and A172 cells, which exhibited intense immunoreactivity with Th9, but little or no immunocytochemical staining with the Th monoclonal antibodies that were used to capture antigen, the levels of thread protein detected by M-IRMA were much lower than those measured in the PNET1 cells (Figure 2). Similarly, Huh7 cells, which manifested no immunocytochemical staining with any of the thread protein monoclonal antibodies, had virtually nondetectable levels of thread proteins in the cellular lysates by M-IRMA. The concentrations of thread protein in the cell lysates were computed from a standard curve generated with purified PTP using Th7 and Th10 as capture antibodies. The results expressed as mean S.D. pg/mg of total protein were as follows: PNET1-13.1 $\pm$ 0.39; PNET2-2.06 $\pm$ 0.10; A172-3.38 $\pm$ 0.37; C6-2.52 $\pm$ 0.22; and Huh7-0.34 $\pm$ 0.05.

Example 3

Characterization of Neural Thread Proteins in Tumor Cell Lines

In Western Blot anaylsis, samples containing 100 μg of protein were fractionated by SDS-PAGE, along with pre-labeled molecular weight standards. The proteins were blotted onto nylon membranes (Immobilon-P transfer membrane, Millipore) using a semi-dry transfer apparatus (Integrated Systems). The membranes were washed in Tris buffered saline (TBS; 10 mM Tris, 0.85% sodium chloride, pH 7.5), and then blocked with TBS containing 3% BSA. The blots were incubated overnight at 4°C with ^{125}-I labeled Th9

monoclonal antibody. Unspecifically bound probe was removed by washing the membranes at room temperature in TBS-BSA 3 x 15 min, and 1 x 30 min. The results were analyzed by autoradiography using Kodak XAR film.

To prepare samples for immunoprecipitation studies, one milliliter samples of cell lysate containing approximately 1 mg/ml of protein were used for immunoprecipitation studies. The lysates were initially pre-cleared with non-relevant antibody (5C3 or antidesmin), and then with Protein A sepharose. Thread proteins were immunoprecipitated using 5-10 μg of Th9 and Protein A sepharose (Sasaki *et al., J. Biol. Chem. 268*:1-4 (1993)). The immune complexes collected by centrifugation were resuspended in buffer containing 2% SDS and 10 mM β-mercaptoethanol, and then subjected to SDS-PAGE under denaturing and reducing conditions (*Id.*). Crude cellular lysates (100 μg protein) were analyzed simultaneously. The proteins were blotted onto Immobilon-P membranes and probed with ^{125}-I labeled (*Id.*) Th9 to detect thread proteins and related molecules. Negative control experiments were performed simultaneously using either monoclonal antibodies to Hepatitis B surface antigen (5C3) or to desmin.

Metabolic labeling experiments were performed using monolayers of cells cultured in 100 mm^2 petri dishes. Prior to labeling, the cells were exposed to methionine- and cysteine-free medium for 2 h. The medium was then replaced with 3 ml of DMEM containing 300 μCi each of [^{35}S] methionine or [^{35}S] cysteine. After labeling for 3 hours, the cells were incubated for various intervals with complete medium devoid radiolabeled amino acids and supplemented with 10 mM methionine. Cell lysates were prepared as described above. Thread proteins were immunoprecipitated using the Th9 monoclonal antibody and protein A sepharose, and the immunoprecipitation products were analyzed by SDS-PAGE and film autoradiography.

For the *in vivo* phosphorylation studies, cells cultured as described for metabolic labeling studies were washed twice with TBS and incubated for 2 h with phosphate-free Dulbecco's MEM containing 10% dialyzed fetal calf

serum. Then the cells were washed with TBS and incubated for 3 h with the same medium containing 400 μCi/ml of [^{32}P] orthophosphoric acid. The cell lysates were analyzed by immunoprecipitation with thread protein, and both positive (p36) and negative (desmin) control monoclonal antibodies, followed by SDS-PAGE.

In order to study the glycosylation state of neural thread proteins, cell culture lysates containing approximately 100 μg or protein were subjected to SDS-PAGE, and the fractionated proteins were transferred to Immobilon-P membranes (Millipore). O- and N-glycans were detected by periodate oxidation followed by biotinylation, and then Western blot analysis with a Streptavidin-alkaline phosphatase probe and NBT/BCIP as the colorimetric substrate. The assays were performed using the GlycoTrack Kit (Oxford Glycosystems, Rosedale, NY) according to the protocol provided by the manufacturer.

Th9-immunoreactive proteins were detected in lysates of PNET1, PNET2, SH-Sy5y, C6, and A172 cells by four different methods: Western blot analysis, immunoprecipitation followed by Western blot analysis, metabolic labeling followed by immunoprecipitation, and SDS-PAGE combined with M-IRMA. Western blot analysis of crude cellular lysates using ^{125}I-labeled Th9 demonstrated ~21 kDa bands in the above cell lines (as indicated by the arrow in Figure 3), but the signal intensity was low. In contrast, in lysates of human pancreatic tissue, the expected 17 kDa uncleaved and 14 kDa cleaved forms of pancreatic thread protein were readily detected by Western blot analysis (Figure 3). Thread proteins were not detected in lysates of human hepatocellular carcinoma cell lines. The strikingly greater abundance of thread proteins in pancreatic tissue compared with neuronal and glial cell lines is consistent with a previous finding of 10^6-fold higher levels of thread proteins in pancreas and pancreatic juice compared with brain tissue and cerebrospinal fluid (Ozturk *et al.*, *Proc. Natl. Acad. Sci. USA 86*:419-423 (1989); de la Monte *et. al.*, *J. Clin. Invest. 86*:1004-1013 (1990); de la Monte *et. al.*, *J. Neurol. Sci. 113*:152-164 (1992); de la Monte *et al.*, *Ann. Neurol.*

32:733-742 (1992)). Although one would expect that thread proteins synthesized by PNET and glial cells are secreted as is the case for PTP and NTP, thread proteins were not detected in the tissue culture medium by Western blot analysis, even after concentrating the medium four- or five-fold by lyophilization.

Th9-immunoreactive thread proteins were more readily detected in PNET and glial cell lines by first immunoprecipitating from the lysates with either Th7+Th10 or Th9, and then performing Western blot analysis using ^{125}I-labeled Th9 (direct) (Figure 3), or unlabeled Th9 with ^{125}I-labeled Protein A (indirect). Both methods demonstrated 21 kDa thread protein-related species, similar to those detected by Western blot analysis. In addition, ~17 kDa bands were also observed in both PNET and glial cells, but the signal was inconsistent and low-level, as determined by Western blot analysis. As negative controls, the Huh7, HepG2, and FOCUS (Lun *et al., In Vitro (Rockville) 20*:493-504 (1984)) human hepatocellular carcinoma cell lines were studied simultaneously under identical conditions, and Th9-immunoreactive proteins were not detected in the cellular lysates.

The molecular sizes of thread proteins present in PNET and glial cells were most prominently demonstrated by metabolical labeling with ^{35}S-methionine or ^{35}S-cysteine, followed by immunoprecipitation using Th9 monoclonal antibody. Monoclonal antibodies to desmin or to hepatitis B surface antigen (5C3) were used as negative controls for immunoprecipitation. In both PNET and glial cell lines, ~26 and ~21 kDa Th9-immunoreactive proteins were detected by SDS-PAGE analysis of the immunoprecipitated products (Figure 4B). In PNET1 cells, the 21 kDa band appeared as a doublet (Figure 4A); the accompanying slightly higher molecular weight species appeared to be less abundant than the dominant band at ~21 kDa. In addition, in both PNET and glial cell lines, there were also ~17 kDa Th9-immunoreactive proteins associated with bands of nearly the same intensity as the ~21 kDa bands. In C6 cells, there were also ~26 kDa, ~14-15 kDa

and ~8 kDa Th9-immunoreactive proteins which were not detected in PNET cells (Figures 4A and 4B, arrows).

The 21 kDa and 17 kDa thread proteins in SH-Sy5y, PNET1, A172, and C6 cells, and their absence in hepatocellular carcinoma cells were also demonstrated by SDS-PAGE/M-IRMA (Figures 5A-5E). Cellular proteins fractionated by SDS-PAGE were eluted from the gels sliced at 2 mm intervals, and assayed directly for thread protein immunoreactivity by M-IRMA using Th7+Th10 as capture antibodies, and ^{125}I-labeled Th9 as the tracer. Despite low levels, two distinct peaks were evident in all neuroectodermal cell lines, but not in Huh7 hepatocellular carcinoma cells assayed simultaneously and in the same manner. The resolution of these gels did not permit distinction of ~17 kDa from ~14-15 kDa proteins which might have been present.

PNET1 and C6 cells were metabolically labeled with ^{32}P or ^{35}S-methionine, and thread proteins were immunoprecipitated from the lysates using Th9 monoclonal antibody (Figure 6). As a negative control, immunoprecipitation studies were conducted using an equal portion of the cellular lysate and monoclonal antibodies to desmin protein (Figure 6, right panel). In the cells labeled with ^{35}S methionine, Th9-immunoreactive bands were detected at ~26 kDa and ~21 kDa (upper arrows), ~17 kDa (lower arrows), and also at ~14-15 kDa (Figure 6). After ^{32}P labeling, only the 21 kDa band was observed by immunoprecipitation with Th9 monoclonal antibody; the other molecular weight species did not appear to be phosphorylated (Figure 6). Phosphorylated Th9-immunoreactive proteins were detected in C6 cells, but not in PNET1 cells, but this might be due to less efficient labeling since PNET1 cells grow slower than C6 cells. No bands in the 14 kDa to 26 kDa range were detected using monoclonal antibodies to desmin for immunoprecipitation (Figure 6). Carbohydrate moieties were not detected in Th9 immunoprecipitated proteins (data not shown).

The highest concentrations of thread protein were measured in subconfluent cultures of PNET1 cells, i.e. during the log phase of growth, and the lowest concentrations in overnight serum-starved cultures (growth arrest)

(Figure 7). Cultures that were 100% confluent also had lower levels of thread protein expression compared with proliferating cultures. Huh7 hepatocellular carcinoma cells (negative control) were simultaneously studied using identical culture conditions, but the levels of thread protein remained low throughout.

Surprisingly, there was no change in the degree of thread protein immunocytochemical staining of PNET cells cultured under these various conditions. However, the degree to which the levels of thread proteins changed by M-IRMA measurement may not have been detectable by immunocytochemistry. Nevertheless, the reduction in cellular thread protein content induced by serum starvation was associated with a change in the phenotype of the cells. When the cells achieved 100% confluence or after they had been subjected to overnight serum starvation, the cell bodies reduced in size, and they exhibited striking changes in the degree and distribution of immunoreactivity for neurofilament protein, GAP-43, and GFAP (Figure 8). In PNET cultures that were 50% confluent, the cells exhibited punctate and often a polar distribution of neurofilament and GAP-43 immunoreactivity, whereas 100% confluent and serum-starved PNET cultures exhibited diffuse perikaryal immunoreactivity for both neurofilament and GAP-43. The punctate immunoreactivity may have corresponded with distribution of neurofilament and GAP-43 in neurites. In contrast, 50% confluent PNET cultures were devoid of GFAP immunoreactivity, while 100% confluent and serum-starved cultures contained conspicuous proportions of GFAP-positive cells. Moreover, the proportion of GFAP-immunoreactive cells was greatest in 100% confluent serum-starved cultures, followed by 50% confluent serum-starved cultures, and then 100% confluent cultures with medium containing 10% fetal calf serum. Therefore, the reduction in thread protein levels measured in PNET cells subjected to overnight serum starvation may have been due to differentiation of the cells toward an astrocytic phenotype. C6 cells and other glioblastoma cell lines exhibited intense immunoreactivity with the Th9 monoclonal antibody, but the levels of thread protein measured by M-

IRMA were often low, possibly due to low-level immunoreactivity with other thread protein antibodies, including Th7 and TH10 (*see* Figures 1A-1J).

Example 4

Cloning of Thread Proteins from Human cDNA Libraries

Human brain cDNA libraries made from 17-18 week old fetal brain (Stratagene, Inc., La Jolla, CA), 2 year-old temporal lobe neocortex (Stratagene), and end-stage Alzheimer's disease cerebral cortex (In Vitrogen; San Diego, CA) were screened using probes generated from a 416 bp DNA fragment corresponding to nucleotides 235-650 of the rat PTP cDNA. The rat PTP cDNA, designated O18, was isolated from a rat pancreatic cDNA library using synthetic 60mer DNA probes corresponding to nucleotides 45-104 and 345-404 of the published sequence (Terazono *et al., J. Biol. Chem. 263*:2111-2114 (1988); Watanabe *et al., J. Biol. Chem. 265*:7432-7439 (1990)). Approximately 2 x 10^6 plaques or colonies from each library were screened with low-stringency hybridization using standard techniques (*see* Sambrook *et al., supra*). Putative clones were plaque/colony purified, and the DNA inserts were sequenced by the dideoxynucleotide chain termination method using T7 polymerase (USB Sequenase; United States Biochemical Corp., Cleveland, OH). The sequences were compared with the Genebank database, and aligned with the nucleic acid sequences of other thread protein cDNAs.

a. CNS Neural Thread Protein cDNA Isolated from Human Fetal Brain Library

A 1.35 kilobase (kb) 1-9a CNS thread protein partial cDNA was isolated in which only a small segment corresponds to an open reading frame, and the remainder, to a 3′ untranslated region (Figure 9). The sequence of

an additional 150 nucleotides was obtained from 5′ anchor PCR amplification products. A second round of 5′ anchor PCR amplification yielded a further upstream 600 bp product (Figure 9A). A portion of the 1-9a cDNA sequence shares significant homology with the 5′ end of the human PTP cDNA and the Reg gene (Figure 10). In addition, the initial 5′ anchor PCR product has 60% homology with the 5′ end of the Reg gene, and 63% homology with Exon 2 of the human Reg gene (Figure 10A). Moreover, probes generated from the 590 bp 5′-end fragment of 1-9a cDNA hybridized with human brain and pancreas mRNA (Figures 12A-12C). The 1-9a sequence is also homologous with the AD2-2 and AD3-4 cDNAs in that at one end of their completed sequences, the overlaps are substantial (Figure 10B).

b. CNS Neural Thread Protein cDNA Isolated from a Two-Year Old Temporal Cortex Library

The HB4 clone is a 593 base pair partial cDNA that was isolated from a 2-year old temporal cortex library. This cDNA contains an open reading frame at its 5′ end and terminates at nucleotide 275. There is a polyadenylation signal beginning at nucleotide 475, and the sequence ends with a poly-A tail (Figure 11A). The deduced amino acid sequence of the partial HB4 clone predicts a protein with a molecular weight of 10.4 kDa, and a pI of 12.1. The HB4 cDNA exhibits 50% overall nucleic acid homology with the human PTP cDNA (Figure 11D), a segment of the human Reg gene (Figure 11E).

c. Isolation of Neural Thread Protein cDNAs from an Alzheimer's Disease Library

Using the O18 rat PTP cDNA probe, four related cDNAs were isolated from an AD brain library. These clones were designated: AD 2-2, AD 3-4, AD 4-4 and AD 16c (also called AD 10-7) (Figures 16A-16S).

The AD 2-2 cDNA is approximately 1.2 kb and it shares significant homology with the 1-9a cDNA, AD 16c, rat PTP cDNA, and Exon 1 of the human Reg gene (Figure 17). The AD 2-2 probe generates a genomic Southern blot pattern similar to that obtained with the AD 3-4 probe. Figure 16E depicts the complete nucleotide sequence of the AD2-2 cDNA clone that was isolated from an AD brain library. Random primer generated probes based on this sequence hybridized with human brain and neuronal samples but not with glial cell lines of with pancreatic RNA.

Figures 16F, 16I, 16J and 16K depict partial nucleotide sequences of the AD3-4 cDNA clones that were isolated from an AD brain library. Rnadom primer generated AD3-4 probes yielded two mRNA transcripts, 1.6 kB and 3.4 kB. These mRNA species are over-expressed in AD brains, with an average of two-fold elevation compared with aged matched controls (N=8).

The AD 3-4 cDNA 1.6 kb clone is identical to another clone isolated at the same time (AD 5-3) (Figure 18A). The AD 3-4/AD 5-3 cDNA exhibits substantial homology with the 1-9a 5' anchor PCR products (Figure 18B), as well as with the human Reg gene and the Gen2a-EP genomic clone (Figure 18B). Southern blot analysis of human genomic DNA with the AD 3-4 probe revealed a pattern similar to that obtained with the AD 2-2 probe.

Figures 16L and 16M depict the partial nucleotide sequence of AD 4-4 which is a 0.8 kb partial cDNA clone which is identical to another cDNA isolated at the same time (AD 3-5). This AD 4-4 clone shares substantial sequence homology with AD 2-2 and 1-9a cDNAs (Figure 19). Figure 16N depicts the complete nucleotide sequence of a partial cDNA clone isolated from an AD brain library. This cDNA hybridized with brain and neuronal cell line mRNA, yielding a single 1.4 kB transcript.

Figure 16O depicts the nucleotide sequence of the 0.5 kb partial cDNA clone AD 16c (also called AD 10-7) that is 72% homologous with AD 2-2, and also aligns with human PTP and the human Reg gene sequences (Figures 20A and 20B).

Figure 16R depicts the complete nucleotide sequence of the AD10-7 clone that was isolated from an AD brain library. Hybridization of Northern blots using either antisense cRNA probes or random primer generated DNA probes detected 2.6, 1.9. 1.4 and 0.9 kB mRNA transcripts in neuronal cells. Neuronal cell lines expressed only the two largest transcripts, while mature adult human brains expressed predominantly the two smallest transcripts, and either very low or nondetectable levels of the 2.6 kB and 1.9 kB transcripts. Using an AD10-7 probe, Northern blot analysis of RNA obtained from human liver, ovary, fallopian tube, colon, stomach, spleen, rectum, thyroid, 12 week placenta and kidney was negative.

Figure 16S depicts the complete nucleotide sequence of the AD16c cDNA clone that was isolated from an AD brain library. Hybridization of Northern blots using random primer generated DNA probes yielded the same results as obtained with the AD10-7 cDNA clone. The AD16c clone shares a 650 bp segment of near identity with AD10-7. In addition, elevated levels of AD16c mRNA were detected in AD brains compared with aged control brains by Northern blot analysis.

Example 5

Analysis of Brain Thread Protein Gene Expression

Thread protein mRNA expression was examined in the following neuroectodermal tumor derived cell lines: central nervous system primitive neuroectodermal tumor cells designated PNET1 and PNET2; HGL-16 and HGL-17 human glioblastoma cells; A172 human glioma cells; C6 rat glioma cells; and SH-Sy5y neuroblastoma cells. In addition, human brain tissue from patients with Alzheimer's disease or no neurological disease (aged controls), and embryonic and postnatally developing rat brain were assayed for thread protein mRNA expression. RNA extracted from human and rat pancreas served as positive controls.

RNA was extracted in 5 M guanidinium isothiocyanate, and then isolated by centrifugation through a cesium chloride step gradient (*see* Sambrook *et al., supra*). RNA was quantified by measuring the absorbance at 260 nm and 280 nm. The thread protein mRNA transcript sizes were assessed by northern blot analysis, and the levels of expression were evaluated by RNA dot blot hybridization. Northern blot analysis was performed by electrophoresing samples containing 15 μg of total cellular RNA through 1% agarose-formaldehyde gels. The RNA was transferred to nylon membrane, cross-linked with ultraviolet light, and hybridized with probes generated from a 600 bp fragment of the 1-9A cDNA clone. The fragment used for hybridization studies contained the regions most homologous with the human PTP cDNA. The probes were labeled with [^{32}P] α-dCTP by the random primer method (Amersham Corporation; Arlington Heights, IL). The blots were hybridized overnight at 42°C with 2 x 10^6 dpm /ml of probe in buffer containing 50% formamide, 5x SSPE, 10x Denhardt's (100x Denhardt's is 2% Ficoll, 2% bovine serum albumin, 2% polyvinylpyrollidine), 0.5% SDS (sodium dodecyl sulfate), and 100 μg/ml of sheared denatured salmon sperm DNA. The membranes were washed in SSPE containing 0.25% SDS using standard methods. Autoradiograms were generated by exposing the membranes to Kodak XAR film at -80°C. The membranes were subsequently stripped of probe and then rehybridized with a synthetic 30mer corresponding to 18s RNA to evaluate sample loading.

Northern analysis of total cellular RNA using probes made from the 1-9a cDNA disclosed two dominant transcripts in central nervous system (CNS) tumor cell lines: one transcript was 1.6 kb, and the other was 0.9 kb (Figure 12A). In addition, in the SH-Sy5y neuroblastoma and PNET1 cell lines, a larger 4.2 kb mRNA transcript was also detected. The 4.2 kb transcript may represent preprocessed mRNA. The same size transcripts were detected in adult (R. Brain) and newborn (NB) rat, but the 0.9 kb transcript was more abundant in the adult brain whereas the 1.6 kb transcript was more abundant in the newborn rat brain. In rat pancreas (R. Panc.), only a 0.9 kB

transcript was detected, corresponding to the size of rat PTP mRNA (Terazono *et al., J. Biol. Chem. 263*:2111-2114 (1988); Watanabe *et al., J. Biol. Chem. 265*:7432-7439 (1990)). mRNA transcripts were not detected in normal liver (NI Liver). Using a probe generated from the 3' region of the 1-9a cDNA, the 1.6 kb, but not the 0.9 kb transcript was revealed (Figure 12B). Using a 30-mer probe corresponding to the most 5'-end of the 1-9a cDNA, the higher molecular weight mRNA transcripts were detected (Figure 12C). The 0.9 kb transcript was also evident with longer exposure of the blot.

Northern analysis of human brain RNA disclosed a dominant 1.6 kb transcript, but also two and sometimes three smaller transcripts of 1.2 kb, 0.9 kb, and 0.8 kb (Figure 13B). In contrast to the findings in cell lines, the 4.2 kb mRNA transcript was seldom observed in adult human brain. Hybridization with human pancreas disclosed a 0.8 kb transcript, corresponding with the size of PTP mRNA. The transcripts detected in human brain and pancreas using 1-9a probes were identical in size to the transcripts observed using PTP cDNA probes.

Dot blot RNA hybridization to 5 μg of total RNA using the 600 bp fragment of the 1-9a cDNA (NTP) demonstrated higher levels of expression in AD, compared with aged control brains (Figure 13A). Rehybridization of the same membrane with a cDNA corresponding to β-actin demonstrated similar loading of RNA in each dot. The observation of elevated levels of 1-9a-related mRNA in AD brain tissue is similar to that reported previously using 60mer probes corresponding to human PTP cDNA (de la Monte *et. al., J. Clin. Invest. 86*:1004-1013 (1990)). The differences between AD and control brains appeared to be due to differences in the levels of the 1.6 kb, 0.9 kb and 0.8 kb transcripts, as shown in Figures 13A and 13B.

The AD-NTP 3-4 cDNA, isolated from the AD library, hybridizes with RNA from neuronal-derived neuroectodermal tumor cell lines, and human brain tissue. In the cell lines, 1.6 kb and 0.9 kb transcripts as observed with the 1-9a probe were detected (Figure 21C). However, in human brain, ~4

kb, 1.6 kb, and 0.9 kb transcripts were detected, and the levels of expression for all three transcripts were higher in AD compared with aged control brains (Figure 21D).

AD 4-4 cDNA probe hybridized only with a 0.9 kb transcript, and only in neuronal cell lines.

Example 6

Direct Cloning and Sequencing of Thread Protein cDNAs from Neuroectodermal Tumor Cell Lines and Alzheimer's Disease Brain

Thread protein cDNAs were cloned directly from PNET1, PNET2, SH-Sy5y, and A172 cells, and from Alzheimer's disease and aged control brain RNA using the 3'- and 5'-RACE methods (Frohman *et al., Proc. Natl. Acad. Sci. USA 85*:8998 (1988); Ohara *et al., Proc. Natl. Acad. Sci. USA 86*:5673 (1989); Loh *et al., Science 243*:217 (1989)). Briefly, RNA was reverse transcribed using oligo-dT primers. For the 5'-RACE reaction, the cDNAs were amplified by polymerase chain reaction (PCR) using a specific 17-mer corresponding to a 5'-region of the 1-9a sequence, and a 17 dT primer. The resulting PCR products were subjected to another round of amplification using another internal but overlapping 5'-end primer, and a specific 3'-17-mer corresponding to a 3' region of the 1-9a sequence. For the 3'-RACE reactions, the cDNAs were first tailed with dCTP using terminal deoxynucleotide transferase, and then they were amplified using a specific 17-mer corresponding to nucleotides 781-797 of the 1-9a clone and dG (17mer). A second nested PCR amplification was performed using a specific 17mer corresponding to nucleotides 766-792 at the 3' end, and dGTP (17mer) for the 5' end. The PCR products were subjected to Southern blot analysis using probes generated from an internal DNA fragment of the 1-9a cDNA clone, and from the O18 rat PTP cDNA clone. The PCR products were gel purified and ligated into pAmpl vectors using uracil deoxytransferase. The

subcloned DNA inserts were sequenced by the dideoxynucleotide chain termination method using T7 DNA polymerase.

CNS thread protein transcripts were detected in neuroectodermal tumor cell lines and in AD human brain tissue by reverse transcription followed by PCR using specific primers corresponding to the 5' and 3' regions of the 1-9a cDNA sequence. Southern blot analysis of the PCR products demonstrated two dominant cross-hybridizing species, 0.8 kb and 1.0 kb (Figures 14A and 14B). In addition, in the SH-Sy5y cells, a larger 1.8 kb PCR product was also detected. In the PNET1, PNET2, SH-Sy5y, and A172 cells, a 0.4 kb PCR product that hybridized with the 1-9a probe was observed. Corresponding with the higher levels of thread protein mRNAs in Alzheimer's disease brains, the hybridization signal was more intense in AD samples compared with aged control samples.

The PCR products generated from the SH-Sy5y cells were subcloned and sequenced. Southern analysis of the cloned fragments exhibited intense hybridization with the 1-9a cDNA, and less intense but definite hybridization with the O18 cDNA (rat PTP) (Figure 14C). The nucleic acid sequence of the SH-Sy5y PCR clone (Sy-NTP) was identical to the 1-9a cDNA sequence.

Example 7

Isolation of Genomic Clones Coding for Human Brain Thread Proteins

A human genomic DNA library was screened using probes made with a 600 bp fragment of the 1-9a human brain thread protein cDNA that was isolated from the two year-old temporal cortex library. The 1-9a cDNA fragment contained a region with 60% nucleic acid sequence homology with human PTP. After colony purification, the putative genomic clones were checked for cross-hybridization with the O18 rat PTP cDNA fragment. *Eco*RI, *Pst*I, and *Eco*RI/*Pst*I restriction fragments that hybridized with both the 1-9a and O18 probes were subcloned into pBluescript II vectors (Promega,

Inc., Madison, WI) and then sequenced by the dideoxynucleotide chain termination method using either T7 polymerase (USB Sequenase) or polymerase chain reaction amplification and Vent polymerase.

Four genomic fragments designated G2-2 *Pst*I, G2-2 *Pst*I-*Eco*RI, G5d-1 *Pst*I, and G5d-1 *Pst*I-*Eco*RI were isolated from a human genomic DNA library (Figures 22A-22D). These genomic fragments all hybridized with both the 1-9a and O18 cDNA probes, and they ranged in size between 1.5 kb and 3 kb. Partial nucleic acid sequence information demonstrated homology between G2-2*Pst*I and the human Reg gene and human and rat PTP cDNAs (Figure 23A); between G2-2 *Pst*I-*Eco*RI and both the Reg gene and rat PTP cDNA (Figure 23B), and also with AD 2-2, AD 3-4, and the 1-9a cDNAs (data not shown); between G5d-1 *Pst*I and the Reg gene and human PTP (Figure 23C); and between G5d-1 *Pst*I-*Eco*RI and Reg gene, human PTP, 1-9a, and AD 4-4.

Example 8

In vitro Expression of the LacZ Fusion Protein and Demonstration of its Relatedness to Thread Proteins

Fusion protein expression in bacteria containing the 1-9a cDNA clone, or one of the four genomic clones was induced with isopropylthio-ß-D-galactoside (IPTG) using standard techniques (Sambrook *et al.*, *supra*). Crude bacterial lysates from induced and uninduced cultures were subjected to SDS-PAGE and Western blot analysis using the Th9 monoclonal antibody to thread protein (Sasaki *et al.*, *J. Biol. Chem. 268*:1-4 (1993)), and ^{125}-I labeled protein A to detect the bound antibody. In addition, bacterial lawns containing cloned DNA were induced to express the fusion protein with IPTG, and replica filters were probed directly with Th9 monoclonal antibody followed by ^{125}-I labeled protein A.

Thread protein immunoreactivity was demonstrated in the bacterial fusion proteins by direct antibody binding to the IPTG-induced colonies (Figures 24A-24D). Thread protein immunoreactivity was detected using a cocktail of Th9, Th7, and Th10 monoclonal antibodies to PTP (Sasaki *et al.*, *J. Biol. Chem.* 268:1-4 (1993), and ^{125}-I labeled Protein A.

Example 9

Relative Levels of AD16c mRNA in AD and Aged Control Brains

Northern blot analysis was performed using an AD16 cDNA probe. The blots were re-probed to detect 18s ribosomal RNA to evaluate loading of RNA in each lane. The unsaturated autoradiograms were subjected to densitometric analysis using a Molecular Dynamics Image Analyzer. The ratios of the AD16c and 18s RNA hybridization signals were plotted for each case, and the results are depicted graphically in Figures 25A and 25B. The mean ratios (relative levels of AD16c) with standard errors are depicted in the smaller right hand graph. The findings confirm that there are elevated levels of AD16c mRNA expression in 6 of 9 AD brains compared to 1 of 6 age-matched controls. The difference between the mean levels is highly statistically significant ($P < 0.005$). Similar results were obtained using AD10-7 probes. Theses results demonstrate that there is a statistically significant increase in levels of expression in AD brains compared to control brains.

Example 10

Preparation of Recombinant AD10-7 Fusion Protein and Detection Thereof With Monoclonal Antibodies

AD10-7 cDNA was ligated into pTrcHIS vectors (In Vitrogen, San Diego) in three different reading frames (two incorrect-A and B, and one correct-C). Bacteria transformed with one of the three plasmids were induced

with IPTG and bacterial lysates were examined for protein expression 0, 1 and 5 hours later. The proteins were fractionated by SDS-PAGE, and Western blot analysis was performed using monoclonal antibodies against the expressed tag protein (T7-tag mouse monoclonal antibodies; Novogen). The blots were developed using the avidin-biotin, horseradish peroxidase method, with diaminobenzidine as the chromogen (Figure 26). A band corresponding to ~45 kDA was detected in bacteria that had been transformed with plasmid DNA which contained AD10-7 ligated only in the correct reading frame (C) (arrow). The same size protein was observed by *in vitro* translation of the AD10-7 cDNA in a rabbit reticulocyte lysate assay system. In both systems, the fusion partner peptide was ~3 kDA, indicating that the cDNA encodes a protein of about ~42 kDA. A ~42 kDA NPT species is routinely detected by Western Blot analysis of neuronal cell lines and of human brain tissue.

Example 11

Demonstration of Neuronal Localization of AD10-7 mRNA Expression by In Situ Hybridization

Sense and antisense cRNA probes were generated from linearized AD10-7 plasmid DNA using SP6 or T7 DNA-dependent RNA polymerase, respectively. The antisense probes hybridized with neuronal cell line mRNA as described above for this clone. The cRNA sense probes, on the other hand, failed to hybridize with RNA by Northern blot analysis. cRNA probes labeled with digoxigenin-UTP were hybridized with human brain tissue sections from early AD. After washing the sections extensively (de la Monte *et al., J. Clin. Invest. 86*:1004-1013 (1990)), the hybridized probes were detected using peroxidase or alkaline phosphatase conjugated monoclonal antibodies to digoxigenin, and the colorimetric reactions were revealed using standard methods. Examination f the sections by brightfield and darkfield microscopy demonstrated hybridization of AD10-7 only in neurons (Fig. 27;

dense aggregates of white grains over cell bodies in (Fig. 27A)). In contrast, and similar to the findings by Northern blot analysis, the *sense* AD10-7 cRNA probes failed to hybridize with brain tissue (Fig. 27B).

Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention, which is defined by the following Claims.

Example 12

Levels of NTP Expression in AD and Other Neurodegenerative Diseases

a. Cellular localization and accumulation of NTP immunoreactivity in AD brains demonstrated by immunohistochemistry

NTP immunoreactivity was detected in histological sections with the high affinity Th9 monoclonal antibody to PTP, the pancreatic form of thread protein. Although the PTP MoAbs cross-react with NTP, NTP is distinct since it differs in molecular mass, and many antigenic epitopes present in PTP are not shared with NTP. Moreover, hybridization studies demonstrated NTP mRNA transcripts in neurons, indicating that NTP is synthesized in brain. NTP immunoreactivity is localized in neuronal perikarya, and in neuropil and white matter fibers. In AD, the density of NTP immunoreactive neurons, and intensity of immunoreactive staining are increased relative to intact aged control brains. Increased neuronal labeling in AD was detected in the cerebral cortex and subcortical nuclei. Within the neocortex, NTP immunoreactivity was primarily distributed in Layers III, V, and VI. Although NTP immunoreactivity was detected in neurons with neurofibrillary tangles or granuole vacuolar degeneration, numerous neurons without overt

neurodegenerative changes also expressed high levels of NTP. NTP immunoreactivity was not distributed in plaques or extracellular neurofibrillary tangles, and dystrophic neurites were not selectively labeled (de la Monte & Wands, *J. Neurol. Sci. 113*:152-164 (1992); Ozturk, M. *et al.*, *Proc. Natl. Acad. Sci. 82*:5627-5631 (1985)).

b. Semiquantitative estimates of NTP expression in AD and other neurodegenerative diseases

A detailed quantitative immunohistochemical analysis of NTP expression was performed using matched paraffin-embedded blocks of different brain regions from patients with AD (N=25), AD plus Parkinson's disease (PD) (N=8), PD, AD plus Down's syndrome (AD+DS) (N=6), or no neurological disease (aged controls) (N=21). In addition, brains with Huntington's disease (N=5) and multi-infarct dementia (N=2) were studied as disease controls. The highest mean densities of NTP immunoreactive neurons were observed in AD and AD+DS, followed by AD+PD, then PD. The AD+PD brains had less severe AD lesions compared with AD, and PD had relatively few AD lesions. In contrast to neurofibrillary tangles and plaques which showed striking regional variation, the densities of NTP immunoreactive neurons were relatively uniform in different neocortical regions. Elevated levels of NTP immunoreactive expression were detected in PD dementia, but the levels were much lower than in AD or AD+PD (de la Monte & Wands, *J. Neurol. Sci. 113*:152-164 (1992)). The finding of high densities of neurofilament immunoreactive dystrophic dendrites in PD dementia, similar to AD, suggested that AD histopathological lesions may have been evolving at the time of death, and thus account for the modestly elevated levels of NTP in these cases. Specificity of the elevated NTP gene expression in AD was corroborated by the absence of increased NTP immunoreactivity in brains with Huntington's disease or multi-infarct dementia. However, in the vicinity of subacute cerebral infarction in both

control and diseased brains, NTP immunoreactivity was strikingly increased in viable appearing neurons, while in the setting of healed infarction, NTP immunoreactive expression was not elevated. Thus, NTP gene expression can be modulated by neuronal injury with attendant reparative or regenerative sprouting.

c. Demonstration of elevated NTP levels in AD brain tissue by a quantitative radioimmunoassay

A highly sensitive three-site monoclonal antibody-based immunoradiometric assay (M-IRMA) was developed to measure NTP concentrations in biological fluids and tissue homogenates. The M-IRMA was developed using the Th7, Th9, and Th10 MoAbs to PTP, which are cross-reactive with NTP in tissue sections and brain homogenates. Briefly, the Th7 and Th10 antibodies, bound to polystyrene beads, were used to capture NTP in brain homogenates or biological fluids. The captured antigens were detected using ^{125}I-labeled Th9 high affinity MoAb. The concentrations of NTP present in the samples were computed from a standard curve generated with different amounts of purified PTP. This highly sensitive assay detected as little as 10 pmol of thread proteins.

Homogenates of fresh frozen brain tissue sampled immediately adjacent to the blocks taken for histological and immunohistochemical staining, were used to measure NTP concentrations. The tissue was homogenized in phosphate buffered saline (0.85% NaCl, 10 mM phosphate, pH 7.4) plus protease inhibitors. The supernatant fractions obtained after centrifugation at 12,000 x g was used to measure NTP concentration by M-IRMA. Note that this gentle extraction procedure excluded membrane bound and insoluble NTP which may have been present in the tissue. However, the Th MoAbs bind to conformational rather than linear epitopes, and immunoreactivity was lost to unpredictable degrees in specimens that had been extracted with denaturing or reducing reagents. Using M-IRMA, significantly high levels of NTP were

detected in AD cerebral tissue compared with corresponding regions of intact aged control brains. In addition, the concentrations of NTP in AD+DS and AD+PD were significantly elevated relative to control and PD. In AD, elevated levels of NTP were detected in all regions of cerebral cortex and in subcortical nuclei, and the degree of increased expression was correlated more with the patterns of NTP immunohistochemical staining in neurons than with the distribution of neurofibrillary tangles and plaques (de la Monte & Wands, *J. Neurol. Sci. 113*:152-164 (1992)).

d. Detection of NTP in cerebrospinal fluid

NTP immunoreactivity was also detected in choroid plexus and ependymal epithelial cells, although corresponding mRNA expression has not been observed. NTP was assayed directly in cerebrospinal fluid (CSF) samples using M-IRMA. To determine the molecular mass of NTP present in CSF and demonstrate its distinctiveness from PTP, which is present in high concentrations in blood, CSF samples containing 100 μg of protein were fractionated by SDS-PAGE, and proteins eluted from the gel fractions were assayed for NTP by M-IRMA as described above. Unlike brain tissue in which several different size NTP-related molecules may be expressed, the only species of NTP detected in clear CSF samples had an Mr of ~21 kD. In contrast, hemorrhagic samples contained a dominant 21 kD peak, and another 14 kD peak, probably corresponding to PTP. In several samples of AD CSF or ventricular fluid, 21 kD NTP molecules could also be detected by immunoprecipitation followed by Western blot analysis using monoclonal or polyclonal Th antibodies.

e. Demonstration of elevated levels of NTP in AD CSF

NTP concentrations were measured in paired postmortem samples of clear ventricular fluid (VF) and temporal lobe neocortex using M-IRMA. The

concentrations of NTP in postmortem ventricular fluid from patients with histopathologically proven AD were significantly elevated compared with the levels in similar specimens from aged control patients. In addition, the concentrations of NTP measured in ventricular fluid were positively correlated with the levels of NTP in cerebral tissue, such that the mean values were nearly identical. Intact aged control and PD brain and VF samples contained low levels of NTP. As observed in histological sections, in the setting of subacute cerebral infarction or non-specific injury, during an interval when regenerative neuronal sprouting would be expected, the levels of NTP measured in both cerebral tissue and VF were also elevated. However, in specimens from patients with remote cerebral infarcts and multi-infarct dementia, no elevation of NTP was detected in either brain tissue homogenates or VF samples (de la Monte, S.M. *et al.*, *Ann. Neurol. 32*:733-742 (1992)).

f. Detection of elevated levels of NTP early in the course of AD dementia

A large clinically-based study was conducted to determine whether the concentrations of NTP in CSF of patients with early manifestations of AD were significantly elevated compared with neurological disease (PD, Multiple sclerosis-MS) and non-demented control patients (back pain, cervical spondylosis, depression, headache, psychosis). NTP concentrations were measured by M-IRMA, and the assays and data analysis were conducted under code. In patients with clinically diagnosed AD, with confirmed follow-up 6 to 10 years later, the concentrations of NTP were significantly elevated compared with both the non-demented and neurological disease control patients (Table 1) (de la Monte, S.M. *et al.*, *Ann. Neurol. 32*:733-742 (1992)). Comparison of antemortem early AD CSF levels with postmortem temporal neocortex and ventricular fluid end-stage AD levels demonstrated striking increases in mean NTP concentration with progression of disease, as opposed to no significant change over the same intervals in aged control

samples. Moreover, in paired samples from 9 patients with AD, the concentrations of NTP in postmortem brain and CSF were 5- to 50-fold higher than in corresponding antemortem CSF samples obtained approximately 6 years earlier. These findings demonstrated that NTP levels were significantly elevated in CSF of individuals with AD, and that NTP levels in CSF may increase strikingly with progression of dementia and neuronal degeneration. Thus, elevated concentrations of NTP in CSF can serve as an in vivo marker for AD neuronal degeneration.

TABLE 1: Elevated NTP Levels in Antemortem CSF in Early AD Dementia

	AD	PD	MS	Control	P-value
No. of Patients	84	45	73	73	
Age (years)	76	61	41	55	< 0.01
Blessed Score	15	5	ND	ND	< 0.001
CSF NTP (ng/ml)	4.2	1.9	1.6	1.3	< 0.001

Example 13

Cloning of the human brain cDNA encoding NTP

a. Strategy for Isolating NTP cDNAs

Using probes prepared with a rat PTP cDNA, a single 1.4 kB mRNA transcript was detected in AD and DS brains (de la Monte, S.M. *et al.*, *J. Clin. Invest. 86*:1004-1013 (1990)). However, low stringency hybridization with either rat or bovine PTP cDNA probes revealed 4 distinct cross-hybridizing NTP transcripts in human brain. To isolate NTP cDNAs, several human brain cDNA libraries were screened using probes derived from the 3′ half of the rat PTP cDNA. Clones were selected for further study

based upon positive Southern blot analysis with probes derived from the 5′ half of rat PTP. In addition to nucleic acid sequence analysis, final clone selection was based upon detection of the appropriate size mRNA transcripts in pancreas and human brain. An incomplete probable NTP cDNA initially isolated from an AD brain library, was then used to re-screen the AD brain, as well as a 17 week human fetal brain library to obtain full-length and other related cDNA clones.

b. Characteristics of the AD7c-NTP cDNA isolated from an AD brain library

The AD7C-NTP clone is a 1.39 kB complete cDNA that encodes a protein with a predicted Mr of 39 kDa (SEQ ID NO:120 and 121, nucleotide and amino acid sequence, respectively). The cDNA contains an AUG start codon, 1140 bp of continuous open reading frame, and a 250 bp 3′ untranslated segment, followed by an AATAAA poly adenylation signal. One unusual feature of the AD7c-NTP cDNA is that it contains a tandemly repeated head-to-tail dimer of a 570 bp sequence within the coding region. The deduced amino acid sequence of the 570 bp sequence is 45% homologous with human PTP. Importantly, there is conservation of the positions of 5 of the 7 Cys residues, a feature that appears to be characteristic of thread proteins (Lasserre, C. *et al.*, *Cancer Res.* *52*:5089-5095 (1992)). The AD7c-NTP protein contains a hydrophobic leader sequence with a potential cleavage at amino acid residue #15, and multiple Ser and Thr phosphorylation motifs. Correspondingly, several NTP molecules expressed in primitive neuroectodermal tumor cell lines (PNET1 and PNET2), and in SH-Sy5y neuroblastoma cells are phosphorylated by insulin stimulation or by activation protein kinase C (see below). The translated AD7c-NTP protein also has numerous hydrophilic domains.

c. Tissue distribution of AD7c-NTP mRNA by Northern blot analysis

Northern blot analysis was performed using 15 μg samples of total RNA extracted from adult human brain, kidney, liver, spleen, gastrointestinal tract (various regions) ovaries, fallopian tubes, uterus, thyroid, lung, skeletal muscle, and pancreas, and from adult rat brain, kidney, liver, spleen, gastrointestinal tract (various regions), testis, thymus, lung, skeletal muscle, and pancreas. Random primer generated [32P]dCTP-labeled DNA probes, prepared with the AD7c-NTP cloned insert as the template, hybridized under highly stringent conditions with RNA from human and rat brain and pancreas. Cross-hybridization signals were not detected in the other organs and tissues. In the pancreas, the AD7c-NTP probes hybridized with 0.9 kB transcripts, corresponding with the size of PTP. In adult human brain, the AD7c-NTP hybridized with 1.4 kB and 0.9 kB mRNA transcripts. In adult rat brain, the AD7c-NTP probes hybridized with 0.8 kB transcripts.

d. Expression of mature and fetal brain forms of AD7c-NTP-related mRNA transcripts

Northern blot analysis demonstrated 5 distinct AD7c-NTP-related mRNA transcripts. Two of the mRNA transcripts (3.2 kB and 1.9 kB) were mainly expressed in fetal brain and neoplastic neuronal cells, e.g. primitive neuroectodermal tumors cell lines, while the other three (1.4 kB, 1.2 kB, and 0.8 kB) were primarily expressed in postnatal developing and mature brains. With increasing age, there was a progressive decline in the steady-state levels of all AD7c-NTP-related mRNA transcripts, and a shift toward exclusive, very low-level expression of the 0.8 kB transcript in the adult rat brain. The major decline in postnatal NTP gene expression was between days 1 and 8, coincident with the reduction in development-associated cortical neuritic sprouting.

e. Demonstration of AD7c-NTP mRNA up-regulated expression in AD brains

AD7c-NTP mRNA expression was examined in AD and aged control brains by Northern blot analysis. RNA was extracted from matched samples of frontal lobe neocortex (Brodmann Area 11), and 15 μg of total RNA were fractionated. Using random primer generated DNA probes, two AD7c-NTP-related mRNA transcripts, 1.4 kB and 0.9 kB were detected in both AD and control brains. Quantitative assessment of the levels of expression was made by volume densitometric analysis of unsaturated autoradiograms. After correcting for differences in sample loading, based upon corresponding 18s ribosomal RNA hybridization signals (obtained by re-probing the blots with a 30mer corresponding to 18s RNA), it was determined that the steady state levels of both the 0.9 kB and 1.4 kB AD7C-NTP mRNA transcripts were elevated in most of the AD brain samples. In addition, the mean level of AD7c-NTP mRNA in AD brains was two-fold higher than in aged control brains ($P < 0.01$).

f. Cellular localization of AD7c-NTP mRNA expression by in situ hybridization

In situ hybridization was used to demonstrate cellular localization of AD7c-NTP-related mRNA transcripts. Antisense and sense cRNA probes were prepared from linearized AD7c-NTP cDNA template, and purified from polyacrylamide gel. cRNA probes labeled with [^{32}P]-UTP were used in Northern blot analysis to demonstrate specificity of hybridization with antisense, and absence of hybridization with sense probes. In situ cRNA probes were labeled with digoxigenin-UTP. Hybridized probes were detected with alkaline phosphatase- or horseradish peroxidase-conjugated anti-digoxigenin antibodies, and BCIP/NBT substrate. AD7c-NTP gene expression was detected in cortical neurons of both AD and control frontal (Brodmann Area 11) and temporal (Area 21) neocortex using antisense cRNA

probes. Hybridization signals were not detected in white matter or glial cells, nor in tissue hybridized with sense cRNA probes (negative control).

g. In vitro translation and expression of the AD7c-NTP clone

Sense and antisense RNA transcripts were incorporated into rabbit reticulocyte lysate in vitro translation assays, and the products analyzed by SDS-PAGE. A single 39 kD protein was generated by translation of sense strand cRNAs. SDS-PAGE analysis of AD7c-NTP recombinant fusion proteins generated in a pTrcHis expression vector (InVitrogen) also demonstrated the translated product to be ~39 kD. Western blot analysis of AD7c-NTP recombinant proteins demonstrated positive immunoreactivity with polyclonal antibodies to PTP, under non-reducing conditions.

h. Polyclonal antibodies to AD7c-NTP are immunoreactive with PTP, and polyclonal anti-PTP is immunoreactive with recombinant AD7c-NTP protein

Rabbit polyclonal antibodies were generated to the AD7c-NTP-pTrcHis recombinant protein. The immunoglobulin fraction was precipitated with ammonium sulfate and dialyzed against PBS. In a radioimmunoassay, the polyclonal antibodies were specifically immunoreactive with the recombinant AD7c-NTP at greater than a 1:100,000 dilution of serum. Western blot analysis was performed under non-reducing conditions since the antibodies may recognize both conformational and linear epitopes. Both anti-AD7c-NTP and anti-PTP exhibited positive immunoreactivity with recombinant AD7c-NTP protein and purified PTP. However, the intensity of cross-reactivity was comparatively low-level for each antibody.

i. Tissue and cellular distribution of anti-AD7c-NTP immunoreactivity

Western blot analysis demonstrated binding of anti-AD7c-NTP with pancreas and brain. The protein recognized in the rat pancreas was 17 kD, the same as detected with anti-PTP. Several low intensity AD7c-NTP-immunoreactive bands were detected in adult rat brain, but the dominant species was ~39 kD. All other rat organs were negative. The same distribution of immunoreactivity was observed with polyclonal anti-PTP, but with brain, the binding intensity was low-level, and most of the bands detected with the AD7c-NTP antibodies were not observed with the PTP antibodies. Immunocytochemical staining demonstrated positive immunoreactivity in PNET cells of neuronal phenotype, and in neurons, neuropil fibers, and axons of mature human brain. Glial cells were not immunoreactive with anti-AD7c-NTP.

j. AD7c-NTP immunoreactivity in AD brain

Studies using postmortem brain tissue demonstrated more abundant and greater intensities of cortical neuron labeling in AD (N=5) compared with intact aged control (N=5) brains. Studies were conducted to examine the molecular sizes of AD7c-NTP-related proteins expressed in AD and aged control brains by either direct Western blot analysis, and by immunoprecipitation followed by Western blot analysis with the same antibodies. 21 kD, 26 kD, and 39 kD AD7c-NTP-related molecules were found in AD (N=6), control (N=7), and infant Down' syndrome (N=1) brains, but higher levels of the 21 kD NTP protein were found in AD relative to control. Although the same size bands were detected with polyclonal anti-PTP, the sensitivity was low, and the relative intensities of the bands were different. For example, the 21 kD and 39 kD NTP molecules were more clearly detected with the AD7c-NTP antibodies. In addition, these

studies demonstrated abnormal size AD7c-NTP-related bands in several AD brains.

k. Characteristics of the large library of MoAbs generated to recombinant AD7c-NTP protein

150 mouse MoAbs were generated to the AD7c-NTP recombinant protein. The hybridoma supernatants were screened by western blot analysis and immunohistochemistry using AD and control brain, and radioimmunoassay of recombinant AD7c-NTP. With another radioimmunoassay, the MoAbs were also screened against PTP. The objectives of these studies were to do the following: 1) select high affinity antibodies that recognize AD7c-NTP-related proteins (NTP), but not PTP; 2) identify antibodies that recognize or bind to neurons in AD brains to a greater extent than in control brains; and 3) determine which antibodies bind to molecules in brain tissue or CSF that are the same size as recombinant AD7c-NTP. These reagents enable specific detection of elevated levels of NTP in brain tissue, and also in CSF. Analysis of 25 representative MoAbs demonstrated several with cross-reactivity between PTP and AD7c-NTP, but most with strong binding only to AD7c-NTP. Western blot analysis confirmed high level binding of all 25 MoAbs with recombinant AD7c-NTP. In addition, 6 antibodies were identified that recognized precisely the same size molecules in brain as detected in the fusion protein, 6 others that recognized slightly high molecular weight molecules in brain, and 10 with low-level or absent binding in brain. The three remaining antibodies recognized completely different size bands in brain compared with AD7c-NTP itself. Immunohistochemical staining studies demonstrated 5 MoAbs with similar high-level binding in AD and control brains, 3 with more intense and widely distributed immunoreactivity in AD brains, 6 with low-level binding in both AD and control brains, and 11 with little r no binding to histological sections of brain. The degree of binding by immunohistochemistry correlated with the findings by Western blot analysis.

l. A radioimmunoassay to measure levels of AD7c-NTP-related proteins in brain tissue and biological fluids (CSF, serum, urine)

A M-IRMA has been developed to specifically measure AD7c-NTP concentrations in brain, CSF, and blood. Empirical studies have been performed with a large matrix of MoAbs linked to a solid phase support to determine which antibodies were suitable for capture of AD7c-NTP antigen in biological fluids. MoAbs were then selected for their high binding capability to recombinant AD7c-NTP bound to a solid phase support after labeling with ^{125}I. MoAbs #2 and #5, used in the M-IRMA, were selected from a panel of 25 MoAbs because of the following characteristics: 1) the antibodies were highly reactive to recombinant AD7c-NTP and not PTP when bound to a solid phase support; 2) the MoAbs specifically stained neurons in AD brains; 3) the MoAbs reacted with a 42 kD species in cell lysates of AD brain by Western blot analysis; and 4) the MoAbs were of the IgG1 isotype, and therefore suitable for labeling with ^{125}I. Finally, competitive inhibition experiments were performed to demonstrate that the MoAbs recognized separate and distinct antigenic determinants on AD7c-NTP molecules.

m. Isolation of AD7c-related cDNAs from an AD brain library

In addition to the AD7c-NTP clone, five related but distinct cDNAs (AD12-1, AD16b, AD19-1, AD11D, AD16c) were isolated from the AD brain library. All 5 cDNAs share either an identical or nearly identical 570 bp sequence with the AD7c-NTP clone. Each of the cDNAs has been subcloned into pTrc-His expression vectors for analysis of the corresponding fusion proteins by SDS-PAGE, Western blot, and M-IRMA. Each of these fusion proteins was immunoreactive with polyclonal AD7c-NTP antibodies. Unique DNA and antibody reagents are made to distinguish expression of the corresponding mRNAs and proteins in neuronal cells and brain tissue. The

same reagents are used to analyze function and evaluate expression of distinct NTP genes in normal and pathological states.

n. Isolation of AD7c-NTP-related cDNAs from a human fetal brain library

Five AD7c-NTP-related cDNA clones (FB1-3c, FB1-6C1, FB2-3C2, FB2-6C1, FB8-3B2) were isolated from a 17 week human fetal brain library. These cDNAs have been partially characterized, and like the AD clones, they also contain an identical or nearly identical 570 bp sequence as described for AD7c-NTP. The 5 FB cDNAs all hybridized to 3.2 kB and 1.9 kB mRNA transcripts in developing rat brains and PNET cell lines. However, the cDNAs exhibited different degrees (intensities) of hybridization with mature brain, and variability with respect to the number (between 1 and 3) of low molecular weight (0.8-1.2 kB) mRNA transcripts detected in immature brain and PNET cells. Sequence data analysis suggests that each of the five FB clones corresponds with at least one of the cDNAs isolated from the AD brain library.

Example 14

The biological functions of NTP with respect to developmental regulation and cell growth in the CNS

a. NTP expression is developmentally regulated

Studies with human brain tissue suggested that NTP expression was developmentally regulated. Using the Th9 MoAb to PTP, and [^{35}S]UTP-labeled cRNA probes generated with the rat PTP cDNA, NTP gene expression was examined in developing and mature rat brains. By in situ hybridization, NTP mRNA expression was detected throughout the CNS at embryonic day 13 (E13). The density of hybridization grains (levels of mRNA expression)

increased throughout development and peaked on postnatal day 8 (P8). By P16, NTP mRNA expression was low-level and similar to adult brains. With regard to NTP immunoreactivity, faint widespread labeling of neuropil fibers, and intense focal labeling of ependymal lining cells were observed in E13 brains. Thereafter, was a rostral-to-caudal wave of neuronal perikaryal NTP gene expression, such that olfactory structures were mainly labeled in E15 and E17 brains, while cerebellar cortical neurons were primarily labeled in P8 and P16 brains. Young adult and aged (>15 mos.) rat brains exhibited low, virtually non-detectable levels of NTP immunoreactivity in scattered cerebral cortex neurons.

b. Quantitative assessment of NTP expression in developing rat brain using AD7c-NTP polyclonal antibodies

Immunohistochemical staining studies using AD7c-NTP polyclonal antibodies yielded results similar to those obtained with PTP polyclonal antibodies. The greater specificity of AD7c-NTP antibodies for brain permitted Western blot analysis and quantitation of NTP expression. Western blot analysis disclosed 6 different size NTP-related proteins in rat brain: 15 kD, 17 kD, 21 kD, 26 kD, 39 kD, and 42 kD. Densitometric scanning of the autoradiographs revealed progressive declines in the levels of several NTP proteins with increasing age. Importantly, like human brain, the 21 kD NTP molecules were expressed at high levels during development, and at low levels in the mature brain. The same was true for the 17 kD and 39 kD species. In contrast, levels of the 26 kD NTP molecules increased with age, while expression of the 42 kD species did not appear to be developmentally regulated.

c. Aberrantly increased NTP expression in Down syndrome occurs prior to the establishment of AD histopathology and dementia

In both control and Down syndrome 19-36 week fetus and infant brains, NTP immunoreactivity was widely distributed in neurons, neuropil fibers, and axons. In control brains, the density of NTP-immunoreactive neurons and the intensity of neuropil fiber labeling reduced substantially within the first decade, generally by 5 or 6 years of age. Thereafter, NTP immunoreactive expression remained low-level in all age groups. Adjacent histological sections were immunostained with a cocktail of MoAbs to neurofilament (SMI31+ SMI32 + SMI34) to delineate the frequency of AD lesions. None of the control brains had neurofibrillary tangles or dystrophic dendrites, but one elderly control had scattered neurofilament immunoreactive plaques. In Down syndrome, neurofilament immunoreactive superficial cortical dystrophic neurites (dendrites) developed and proliferated during early childhood, probably representing one of the earliest histopathological manifestations of AD neuronal degeneration. Neurofibrillary tangles were first detected between ages 10 and 20 years, while neurofilament-immunoreactive plaques were initially detected in the fourth decade of life.

In developing Down syndrome brains, NTP immunoreactive expression in neuronal perikarya and neuropil fibers increased along with the large-scale proliferation of superficial neurofilament-immunoreactive dystrophic dendrites, and the appearance of neurofibrillary tangles. With increasing age and evolution of AD lesions, NTP expression further increased in Down syndrome. The increased NTP immunoreactivity was not restricted to neurons with neurofibrillary tangles or granuole vacuolar degeneration, nor was it localized in plaques. Increased NTP gene expression in Down syndrome brains begins at least two decades prior t the establishment of clinical and histopathological AD. Thus NTP up-regulated gene expression is an early marker of AD neuronal degeneration, age and development of AD lesions.

d. Demonstration of distinct NTP molecules in developing and mature human brains

The molecular sizes of the NTP molecules expressed in brain were determined by SDS-PAGE fractionation of 100 μg samples of protein, followed by radioimmunoassay (M-IRMA) of proteins eluted from gel slices. The results were graphed with respect to distances migrated by simultaneously analyzed molecular weight standards. Regardless of age, the dominant NTP species detected in brain was 21 kDa. In AD, with or without underlying Down syndrome, small 39 kDa and 26 kDa NTP peaks were also detected. In both control and Down syndrome infant brains, a prominent 17 kD NTP peak was also detected.

e. Development of an in vitro model to examine NTP expression during growth and differentiation

Of the human primitive neuroectodermal tumor (PNET) cell lines--PNET1, PNET2, and SH-Sy5y--PNET1 and PNET2 were of CNS PNET origin, while SH-Sy5y cells were derived from a neuroblastoma. All primary human PNET brain tumors have been found to express NTP. The 3 PNET cell lines used were demonstrated to have neurofilament, synaptophysin, and GAP-43 immunoreactivities, confirming their neuronal phenotypes. In addition, PNET2 and SH-Sy5y cells undergo neuronal differentiation with neuritic sprouting, increased synaptophysin expression, and decreased vimentin expression following treatment with retinoic acid, insulin, or phorbol esther myristate (PMA). In contrast, the PNET1 cells are highly primitive, and fail to exhibit growth factor mediated cell growth or differentiation.

f. Characterization of NTP expression in PNET cells

In all three PNET cell lines, five different NTP species with Mr's of 39-42 kD, 26 kD, 21 kD, 18 kD, and 15 kD were detected by (1) direct

Western blot analysis, (2) metabolic labeling followed by immunoprecipitation, or (3) radioimmunoassay (M-IRMA). NTP gene expression was detected using Th polyclonal or monoclonal antibodies to PTP. Unlike adult human brain where the dominant NTP species detected was 21 kD, in PNET cells, the 39 kD, 18 kD, and 15 kD NTP molecules were most abundant, while the 21 kD and 26 kD were expressed at low or non-detectable levels. Pulse-chase and metabolic labeling studies demonstrated that the 18 kD and 26 kD species were probably derived from other NTP molecules. Additional studies demonstrated phosphorylation of the 39 kD, 26 kD, 21 kD, and 18 kD NTP molecules. Moreover, tyrosyl phosphorylated residues were detected in the 39 kD and 18 kD NTP molecules by Western blot analysis of the immunoprecipitated proteins. Finally, after stimulation with either PMA or insulin, a rapid supershifts in NTP molecular mass from 15 kD to 18 kD with incorporation of [^{32}P] orthophosphate as observed in SH-Sy5y and PNET2 cells. Glycosylation of NTP has not been detected. Therefore, at least some of the NTP molecules are likely to be phosphoproteins.

^{35}S-Met-labeled NTP molecules were immunoprecipitated using PTP Th MoAbs. Rapid labeling of the 39 kD, 21 kD, and 15 kD proteins, with subsequent appearance (within 10-30 min) of 26 kD, and 18 kD NTP species occurred. Thus some NTP molecules can be derived rather than synthesized de novo.

g. Insulin modulation of NTP expression

Insulin is an important mediator of growth and differentiation in CNS neurons. Insulin stimulated differentiation of PNET2 cells was associated with rapid (within 10 minutes) but transient increases in the levels of the 39 kD, 18 kD and 15 kD NTP species, followed by sustained increases in synthesis and steady state levels of all five NTP species. In contrast, the failure of insulin to induce differentiation of PNET1 cells was associated with absent insulin modulation of NTP. Analysis of the signal transduction pathways

demonstrated that the insulin-induced up-regulation of NTP molecules in PNET2 cells was mediated through phosphorylation of the insulin receptor substrate-1 (IRS-1) and the insulin receptor β subunit (IRβs) itself. In PNET1 cells, the lack of insulin responsiveness was associated with impaired insulin-mediated tyrosyl phosphorylation of IRS-1, but normal insulin receptor phosphorylation. Correspondingly, the insulin-stimulated association between PI3 kinase and phosphorylated IRS-1 was also impaired in PNET1 cells. In essence, impaired insulin-mediated tyrosyl phosphorylation of IRS-1 in PNET1 cells halted activation of the insulin signal transduction cascade, and subsequent events leading to modulated gene (NTP) expression. PNET1 cells lacked insulin responsiveness and failed to phosphorylate IRS-1, but insulin receptor levels and tyrosyl phosphorylation (PY) of the β-subunit were intact. PNET2 cells responded to insulin stimulation with phosphorylation of IRS-1, up-regulation of NTP, and neuronal differentiation. The results were confirmed by absent association between PI3 kinase and IRS-1-PY in PNET1 cells after insulin stimulation.

h. Phorbol esther myristate (PMA) and retinoic acid (RA) modulate NTP expression and neuronal differentiation

PMA and RA induced PNET2 and SH-Sy5y cells to differentiate into neurofilament-positive, GAP-43-positive, vimentin-negative cells with fine interconnecting neuritic processes. Following PMA stimulation, there was immediate phosphorylation of the 15 kD NTP species, with a supershift in molecular mass to 18 kD. After 12 hours of PMA or RA stimulation, synthesis of the 21 kD and 26 kD NTP species increased four- to five-fold, followed by intracellular accumulation of these same molecules. At the same time, housekeeping gene expression, e.g. GAPDH, was not affected. PMA and RA treatment also resulted in a shift from the perikarya to neuritic process localization of NTP immunoreactivity. Since the effects of PMA stimulation were mimicked by phosphatidylserine plus diolein treatment, and blocked by

inhibitors of protein kinase C, expression of the 21 kD and 26 kD NTP species can be modulated through the protein kinase C cascade.

i. In vitro stimulation studies

Neuritic sprouting and neuronal differentiation were induced in PNET2 and SH-Sy5y cells by insulin, PMA, or RA stimulation. Insulin-mediated neuritic growth was associated with increased expression of the fetal brain and PNET-dominant forms of NTP (15 kD and 18 kD). In contrast, the PMA- and RA-induced neuritic sprouting modulated expression of the 21 kD and 26 kD NTP species, which are primarily expressed in the mature brain, and accumulated in AD brains. Thus, expression of the immature or fetal forms of NTP are regulated by mechanisms and growth factors distinct from those involved in modulating expression of the 21 kD and 26 kD NTP molecules. Therefore, expression of fetal NTP molecules/genes can be mediated through the IRS-1 cascade, whereas expression of adult brain/AD-associated NTP genes can be regulated mainly through protein kinase C pathways.

Example 15

AD7c-NTP gene expression in postmortem brain tissue from a large number of patients with AD, other neurodegenerative diseases, e.g. Parkinson's Disease, and no neurological disease (aged controls)

a. Source of tissue

Matched snap-frozen, and adjacent formalin-fixed paraffin-embedded blocks of cerebral tissue from different brain regions (Table 2) is used to analyze AD7c-NTP gene expression. All specimens are obtained from the Alzheimer's Disease Research Center (ADRC) Brain Bank located at the Massachusetts General Hospital (MGH). Additional fresh tissue is continually harvested by the ADRC Brain Bank. The histopathological sections are

reviewed for all cases. Routine neuropathological evaluation includes luxol fast blue-hematoxylin and eosin, Bielchowsky silver, and Congo red staining, and ubiquitin and neurofilament immunostaining to detect neurodegenerative lesions in paraffin-embedded sections.

TABLE 2: Postmortem Samples for AD7c-NTP Gene Expression Studies

Diagnosis	Total Number of Cases		
	Frozen Tissue*	Paraffin Blocks**	Ventricular Fluid
AD	50	50	50
PD	10	10	5
DLBD	8	10	0
Pick's	4	8	2
ALS	0	4	0
Down + AD	7	8	4
Aged control	50	50	50

Frozen Tissue Ventricular Fluid Assays	Paraffin Tissue Section Studies
RNAse Protection or RT/PCR Western blot analysis M-IRMA	*In situ* hybridization Immunohistochemistry

* Brodmann Areas: 21, 11, 40, 17; amydgala; midbrain, striatum, cerebellar cortex, s. cord.

** Brodmann Areas: 21, 11, 24, 40, 17; amygdala, hippocampus, midbrain, cerebellum, s. cord.

b. Tissue Processing

Frozen tissue blocks (approximately 2 x 2 x 0.5 cm) are divided for RNA and protein extraction. RNA is extracted by the Chomczynski-Sacchi ne-step guanidinium isothiocyanate/phenol method (Chomczynski & Sacchi,

Anal. Biochem. 162:156-159 (1987)) using a commercially available reagents, e.g. RNAzol or TRIzol. The integrity of RNA is assessed by Northern blot analysis using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA, and 18s synthetic 30mer (Enoch, T. *et al.*, *Mol. Cell. Biol. 6*:801810 (1986)) probes. Completely degraded samples are not used. Protein assays are performed with tissue homogenates prepared in 5 volumes of PBS plus protease inhibitors (Sasaki, Y. *et al.*, *J. Biol. Chem. 268*:3805-3808 (1993)). The supernatant fractions obtained by centrifugation at 12,000 x g for 30 minutes at 4°C, and pelleted proteins solubilized in 1% SDS-containing buffer are used for Western blot analysis and M-IRMA. Previous studies demonstrated that a substantial portion of thread proteins can be contained in the pellet fractions due to reduced solubility, particularly in AD brains. Protein content is determined by the Lowry (Lowry, O.H. *et al.*, *J. Biol. Chem. 193*:265-275 (1951)) or BioRad colorimetric assay. Paraffin-embedded histological sections of tissue adjacent to the blocks processed for the quantitative RNA and protein studies, are used for immunohistochemistry and in situ hybridization studies.

c. Western blot analysis

Western blot analysis is employed to determine the size and relative abundance of the AD7c-NTP proteins present in brain homogenates. Polyclonal AD7c-NTP antibodies is utilized in these studies in order to simultaneously detect all NTP species. Both supernatant (soluble) and pellet (insoluble) fractions are analyzed. 100 μg samples of protein is fractionated in Laemmli SDS-PAGE gels, transferred to Imobilon (nylon) or ECL Hybond membranes, and probed for AD7c-NTP expression using rabbit polyclonal antibodies generated to the recombinant fusion protein (Harlow & Lane, *Antibodies. A Laboratory Manual*, Cold Spring Harbor Laboratory (1988) Cold Spring Harbor, NY). Antibody binding is detected with horseradish peroxidase conjugated goat anti-rabbit IgG, and enhanced chemiluminescence

reagents (Amersham). Pre-stained molecular weight standards is included on each gel. In addition, Western blots generated with different amounts (between 0.1 μg and 10 μg) of purified recombinant AD7c-NTP protein in each lane, is probed simultaneously to provide a basis for normalizing data among different experiments. The autoradiograms are subjected to volume densitometric scanning to quantitate the steady-state levels of AD7c-NTP protein expressed.

d. Immunohistochemical Staining

Paraffin sections (Table 2) are de-waxed in xylenes and re-hydrated through graded alcohol solutions. AD7c-NTP immunoreactivity is assessed using rabbit polyclonal antibodies generated to the recombinant fusion protein. Antibody binding is detected by the avidin-biotin horseradish peroxidase complex (ABC) method using the Vectastain-Elite kit according to the manufacturer's protocol. Immunoreactivity is revealed with diaminobenzidine. The sections are counterstained with hematoxylin, dehydrated through graded alcohols, cleared in xylenes, and preserved under coverglass with permount. The sections are processed in large groups, adhering rigidly to the incubation conditions. The results are analyzed under code to determine the presence and distribution of AD7c-NTP immunoreactivity.

e. In situ hybridization

Cellular localization of AD7c-NTP gene expression is assessed by in situ hybridization using paraffin-embedded tissue (Table 2). The sections are prepared and prehybridized. Antisense and sense (negative control) digoxigenin-UTP labeled cRNA probes are generated from AD7c-NTP plasmid linearized with KpnI or XhoI, using T7 or SP6 DNA-dependent RNA polymerase, respectively. The probes are gel purified to remove free nucleotides. The sections are hybridized overnight at 50°C with 200 ng/ml

of probe (Lee, M.-E. *et al.*, *J. Clin. Invest.* *86*:141-147 (1990). After extensive washing and RNAse A digestion to destroy single-stranded RNA, hybridized probes are detected with alkaline phosphatase conjugated anti-digoxigenin, and the antibody binding are revealed with a suitable chromogen, e.g. BCIP/NBT. Sections counterstained with hematoxylin and preserved with aqueous mounting medium are examined and photographed by brightfield and darkfield microscopy. Adjacent sections are evaluated for intactness of RNA by performing in situ hybridization with cRNA probes corresponding to GAPDH.

f. RNAse Profection Assay

RNAse protection assays are used to examine levels of AD7c-NTP mRNA expression because, compared with Northern blot analysis, this technique is relatively insensitive to the small degrees of RNA degradation which frequently exist in postmortem tissue. Samples of 5 or 10 μg of total RNA are hybridized with gel purified antisense [^{32}P]UTP-labeled cRNA probes (Current Protocols in Molecular Biology. Ausubel *et al.* Eds., John Wiley & Sons, New York, 1994) corresponding to nucleotides 670 to 910 of the AD7c-NTP cDNA. As a positive control, the same samples are simultaneous hybridized (in the same assay tubes) with identically labeled 316 bp antisense cRNA probes corresponding to exons 5-8 of the GAPDH gene (Sabath, D. *et al.*, *Gene* *91*:185-191 (1990)). After hybridization, single-stranded RNA are digested with RNAse A and RNAse T1 (Current Protocols in Molecular Biology. Ausubel *et al.* Eds., John Wiley & Sons, New York, 1994, and the protected probe fragments analyzed on denaturing polyacrylamide gels. tRNA and human infant brain RNA are used as negative and positive controls, respectively. Non-saturated autoradiograms are subjected to volume densitometric analysis to quantitate the hybridization signals.

g. Alternative method for examining levels of AD7c-NTP mRNA Expression

Reverse transcription/polymerase chain reaction amplification of RNA has been successfully used to study gene expression. The advantage of RT/PCR over RNAse protection is that multiple genes can be studied simultaneously, utilizing only 1 μg of total RNA as starting material. Low yields of RNA have been problematic in the past, particularly with respect to AD brain tissue. RNAse protection assays are performed on samples with abundant RNA yields, and RT/PCR are performed with all samples. Messenger RNA are reverse transcribed using oligo-dT and random oligonucleotide primers. The cDNAs are amplified with primers that flank the sequences contained between nucleotides 670 and 910 of AD7c-NTP. The results are analyzed using 1-3% Nusieve agarose gels and ethidium bromide staining. In addition, to confirm the authenticity of PCR products, Southern blot analysis is performed using either [^{32}P]dATP or [fluorescein]dATP end-labeled oligonucleotide probes corresponding to internal sequences of the amplified DNA segment. The minimum number of PCR cycles required to detect AD7c-NTP is determined to ensure the amplified products do not reach saturation.

h. Construction of a two- or three-site monoclonal antibody based immunoradiometric assay (M-IRMA) to measure AD7c-NTP concentration

Two- or three-site forward sandwich M-IRMAs are used to measure AD7c-NTP concentrations. An assay using the #5 and #2 antibodies which specifically recognize NTP (not PTP) exhibits greater degrees of binding to AD compared with aged control brains by immunohistochemical staining. The configuration of the prototype two-site M-IRMA is as follows: the #2 MoAb serves as the capture antibody bound to a solid-phase support (0.25" polystyrene beads). After incubating the coated beads with brain tissue

homogenates or CSF, the captured AD7c-NTP proteins are detected with ^{125}I-labeled #5 MoAb, which serves as a tracer. The radioactivity remaining on the beads after extensive washing is measured in a gamma counter. The signal-to-noise ratios are calculated, and the concentrations of AD7c-NTP in the samples, determined from a linear standard curve constructed with different amounts of recombinant AD7c-NTP protein. Immunoreactivity is measured in 200 μl volumes of diluted tissue extract, CSF, or serum. The lower limit of sensitivity is between 1 and 10 pg per ml of purified recombinant AD7c-NTP protein.

Brain protein extracts corresponding to the supernatant (soluble) and pellet (insoluble) fractions, and samples of postmortem ventricular fluid are assayed in quadruplicate at 1:10, 1:50, and 1:100 dilutions to generate S:N ratios within the linear range of the standard curve. The AD7c-NTP protein concentrations are measured in brain tissue by M-IRMA, using the forward sandwich assay described. Other configurations with one- or two-site MoAb capture are also used.

i. Characterization of additional anti-AD7c-NTP MoAbs

The M-IRMA is optimized in terms of sensitivity and specificity for detecting NTP molecules that accumulate in AD brain tissue. The hybridomas are screened by evaluating the extent of immunoreactive binding in solid phase support immunoassays, Western blot analysis, immunohistochemical staining, as described above with the first 25 AD7c-NTP MoAbs. For the solid phase support immunoassay, 50 ng of recombinant AD7c-NTP protein are bound to polypropylene surfaces in 96-well plates. Hybridoma supernatant are reacted, and antibody binding detected using ^{125}I-labeled goat anti-mouse IgG. Specificity for AD7c-NTP is assessed by demonstrating absent binding to purified PTP, and significant binding above background levels generated with nonrelevant hybridoma supernatant. Indirect Western blot analysis are performed using recombinant AD7c-NTP protein and human AD brain

homogenates, and immunoreactivity is detected with ECL reagents. Western blot analysis permits rapid comparison of the relative sizes of the recombinant protein with the AD7c-NTP-related molecules expressed in brain. Immunohistochemical staining of Brodmann Area 11 in the frontal lobe using neet hybridoma supernatant is performed to demonstrate the distributions and relative levels of AD7c-NTP MoAb immunoreactivity in corresponding sections of AD and control brains. Immunohistochemical screening permits the identification of AD7c-NTP MoAbs with specific immunoreactivity in neurons, and greater degrees of binding in AD compared with aged control brains.

Example 16

Use of M-IRMA to compare the levels of AD7c-NTP protein in AD, aged control, and neurological disease control samples of postmortem ventricular fluid and antemortem CSF

a. Source of specimens

Postmortem ventricular fluid samples from patients with AD, PD, Down syndrome, and normal aging have been obtained from the ADRC-MGH brain bank (Table 2). Approximately 400 clinical cerebrospinal fluid (CSF) samples (Table 3) from patients with AD, PD, multiple sclerosis, non-dementing psychiatric disease (controls), minor neurological ailments, e.g. back pain (controls), or multi-infarct dementia have also been banked. These samples represent discarded clinical material from previously approved studies, or samples obtained for diagnostic purposes. The AD and PD samples were obtained from patients enrolled in the ADRC, and therefore long-term clinical follow-up, including autopsy in many instances, is available. The psychiatric case samples were obtained from patients hospitalized at the Bedford VA Hospital. These samples also represent discarded specimens

from previously approved clinical studies, and long-term clinical follow-up data has already been obtained and incorporated into a computerized database.

TABLE 3: Clinical CSF Samples for M-IRMA

Diagnosis	No. of Cases	Dementia
AD	154	Yes
PD	56	Yes
Multiple Sclerosis	75	No
Multi-infarct dementia	5	Yes
Psychiatric	28	No
Control	94	No

b. M-IRMA Assay to measure AD7c-NTP in ventricular fluid and CSF samples

The samples are diluted 1:10, 1:50, and 1:100 in PBS/BSA, and assayed in quadruplicate for AD7c-NTP as described above. Samples yielding results with signal:noise (S:N) ratios that do not fall within the linear range of the standard curve generated with purified recombinant AD7c-NTP protein are re-assayed at higher or lower dilutions as required. Results are expressed with respect to volume and protein concentration.

c. Analysis of the size of AD7c-NTP present in ventricular fluid and CSF

The molecular size of the AD7c-NTP molecules present in CSF or ventricular fluid is determined by Western blot analysis. These studies determine whether the levels f the 21 kD or another NTP species are increased in AD compared with aged control CSF and ventricular fluid specimens. Samples containing 100 μg of protein are fractionated in Laemmli

SDS-PAGE gels with molecular weight standards. For comparison with results obtained using polyclonal antibodies, the Western blots are re-probed with the tracer (detection) AD7c-NTP MoAb used in the M-IRMA. Additional planned efforts to generate MoAbs that bind to specific and unique NTP molecules will abrogate the need to perform Western blot analysis, in order to interpret abnormally elevated levels of AD7c-NTP. Complete analysis of samples will be possible with a series of rapid and simple M-IRMAs.

Example 17

Analysis of levels of AD7c-NTP in serum of AD

Approximately 100 AD antemortem serum samples, representing discarded specimens from previously approved clinical studies, are available. In addition, approximately 250 samples of serum from normal individuals, obtained from previously approved, unrelated studies are also available. Finally, postmortem serum from patients with confirmed AD has been banked by the ADRC-MGH, and is available. Studies are conducted to determine whether AD7c-NTP molecules are detectable in serum by Western blot analysis. Although some of MoAbs generated to recombinant AD7c-NTP cross-react with PTP, which is abundantly present in serum, the antibodies selected for M-IRMA exhibit no detectable binding to purified PTP at concentrations as high as 1 mg/ml. The objective is to determine whether AD7c-NTP can be measured in serum samples, and whether elevated levels of AD7c-NTP detected in postmortem ventricular fluid, brain tissue, or CSF are detectable in paired serum samples from the same individuals. Another objective is to determine whether the levels of AD7c-NTP are elevated in sera from probable and definite AD patients for whom CSF samples are not available. Sera diluted 1:10, 1:50, 1:100, and 1:250 in PBS/BSA are assayed for AD7c-NTP by M-IRMA.

Example 18

Additional AD and fetal brain NTP cDNAs

Data suggest that the NTP molecules expressed in such non-AD related diseases, are 15 kD or 17 kD, rather than 21 kD in size. A family of NTP cDNAs was isolated from AD brain, and 17 week human fetal brain (FB) libraries. Analysis of the nucleic acid sequences indicates clear regions of extreme homology or identity, as well as unique domains in each clone.

Both strands of each clone are sequenced by the dideoxynucleotide chain termination method using T7 polymerase and custom oligonucleotide primers, with incorporation of deazo-G analogue nucleotides to help circumvent compression artifacts. The nucleic acid and amino acid sequences are compared with the AD7c-NTP cDNA, and with the published sequences of non-neural thread proteins (PTP and HIP).

mRNA expression of the different NTP cDNAs is examined using RT/PCR technology with primers designed to amplify 200 to 500 bp unique sequences contained within each cDNA. cDNA fragments amplified from plasmid templates are directionally subcloned into pGEM vectors to generate digoxigenin-labeled cRNA probes for in situ hybridization studies. To quickly assess potential function, RT/PCR and in situ hybridizations are conducted on a battery of test samples (Table 4). NTP gene expression is quantitated by Southern blot analysis of PCR products, relative to the levels of simultaneously amplified GAPDH.

TABLE 4: Samples for Surveying Expression of Different NTP Genes

Mature Brain	*Developing Brain*	*PNET2 and SH-Sy5y cells*
1. AD (N=4)	1. 2nd trimester fetus (N=2)	1. overnight serum starved
2. normal aging (N=4)	2. 3rd trimester fetus (N=2)	2. proliferating
3. Acute stroke + intact tissue (N=3)	3. infant (N=2)	3. retinoic acid-induced differential
4. Remote stroke + intact tissue (N=2)	4. 5 year old (N=2)	4. vehicle-treated negative control
	5. 16 year old (N=1)	

Methods to Examine NTP Gene Expression:

RNA Studies

In situ hybridization
RT-PCR or RNAse Protection

Protein Methods

Immunocytochemistry
Western Blot analysis
Metabolic Labeling/Immunoprecipitation (cell culture studies only)

The PCR amplified cDNA fragments are subcloned into pTrc-His vectors (InVitrogen) to generate recombinant proteins for immunization and analysis of immunoreactivity. The recombinant proteins are purified by metal chelate affinity chromatography. The purified and concentrated (Centricon filter) recombinant fusion proteins are used to generate monoclonal and polyclonal antibodies for examining protein expression in brain and PNET cell lines.

DNA sequence analysis suggests a need to generate polyclonal and MoAbs to 3 or 4 distinct recombinant truncated fusion proteins to distinguish among the different NTP molecules expressed in brain and PNET cell lines. Polyclonal antibodies are generated first and used to study gene expression. Rabbits are immunized with purified AD-NTP or FB-NTP truncated proteins (described above) (Harlow & Lane, *Antibodies. A Laboratory Manual*, Cold Spring Harbor Laboratory (1988) Cold Spring Harbor, NY). Pre-immune

serum, and serum obtained after the second boost are assayed for NTP-specific immunoreactivity by Western blot analysis and solid phase immunoassay using different amounts of recombinant protein, and with AD7c-NTP, as well as the other NTP truncated proteins as negative controls. The resulting polyclonal antibodies are used to examine the levels and cellular and tissue distributions of immunoreactivity by Western blot analysis and immunocytochemistry using a battery of testing materials (Table 4).

All publications and patent applications mentioned in this specification are indicative of the level of skill of those in the art to which the invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in their entirety.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The General Hospital Corporation

(ii) TITLE OF INVENTION: Neural Thread Protein Gene Expression and Detection of Alzheimer's Disease

(iii) NUMBER OF SEQUENCES: 121

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: (to be assigned)
(B) FILING DATE: 14-NOV-1995
(C) CLASSIFICATION:

(vii) PRIORITY APPLICATION DATA:
(A) APPLICATION NUMBER: 08/340,426
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(C) CLASSIFICATION:

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(C) REFERENCE/DOCKET NUMBER: 0609.384PC02

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGATTCCAA CAGACCATCA T 21

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCAACAGACC ATCATTCCAC C 21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCAAACCGAT TCCAACAGAC C 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCTGGGCAAC AAGAGCGAAA ACTCCATCTC 30

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATCGCTTGAA CCCGGGAGGC GGAGGTTGCG 30

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGGGAGGCTG AGGCAGGAGA ATCGCTTGAA 30

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(C) OTHER INFORMATION: /label= misc_feature
/note= "N" represents a nucleotide linkage of 0-10 bases which links nucleotide "C" at position 21 and nucleotide "C" at position 23.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TACTACCAGA CAACCTTAGC CNCCGATTCC AACAGACCAT CAT 43

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(C) OTHER INFORMATION: /label= misc_feature
/note= "N" represents a nucleotide linkage of 0-10 bases which links nucleotide "T" at position 21 and nucleotide "T" at position 23.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCGATTCCAA CAGACCATCA TNTACTACCA GACAACCTTA GCC 43

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both

(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(C) OTHER INFORMATION: /label= misc_feature
/note= "N" represents a nucleotide linkage of 0-10 bases whi
links nucleotide "C" at position 21 and nucleotide "C" at position

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCACCTTACT ACCAGACAAC CNCCAACAGA CCATCATTCC ACC 43

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(C) OTHER INFORMATION: /label= misc_feature
/note= "N" represents a nucleotide linkage of 0-10 bases whic
links nucleotide "C" at position 21 and nucleotide "C" at position

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCAACAGACC ATCATTCCAC CNCCACCTTA CTACCAGACA ACC 43

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(C) OTHER INFORMATION: /label= misc_feature

/note= "N" represents a nucleotide linkage of 0-10 bases which links nucleotide "C" at position 21 and nucleotide "C" at position 23.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCAGACAACC TTAGCCAAAC CNCCAAACCG ATTCCAACAG ACC 43

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(C) OTHER INFORMATION: /label= misc_feature
/note= "N" represents a nucleotide linkage of 0-10 bases which links nucleotide "C" at position 21 and nucleotide "C" at position 23.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCAAACCGAT TCCAACAGAC CNCCAGACAA CCTTAGCCAA ACC 43

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1443 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGCTGCGCCC AGGCTGGCTC TGGAAAGCCT GTGCGGTCCT GGCAGGAAGC CCGGCCCGTG 60

GAGCAGGTTT TCGTTCTGCT TCAGCAATAA ATAAGGGTGA CCACAGGGAC TTTGCTTTTG 120

GTTTCCTTTC CTGTGAAAAG GTTGGTTTTA AAGTGAGATA CACTTTTCCG TAGAACAAGT 180

GTTCTATCTT TAAAAACCCA AATTGCAGCA CCGTGGATTA CTGGTCTCAG AACAACTCAT 240

TGCGCATCAG ATTTGACTCT CTGATTTTCT GTCTATTGGC CAAATTGCCC TTTAACTGCA 300

CCTGAATCCT TTGTGTACTG ATGCCTTTGA GCTGGGCACC TTGGGAGAGT GTTGTGTTGC 360

TGTTTACGGT TCTTCCTTCC CCTTGCTAAT TACAGTCTCT GGTGCCCAGC AAGCCCCTTT 420

GGCTTCCTTC CGTGACTGGT CACGTTGTCT GCCTGGGCTC AGCGTGGACC TGCCCCATGC 480

TGCAGAACCT GGCCTCACCT GGACTTTTAC TAGAATTGCC AGCTTCTCAA CTTAGCAGAT 540

CATCACTCAT GCGGGCACAA GCAAAGATCA ACACTTTCTT TTTTGGTAAG CTTGAGTTTT 600

ACAAGTTATT TTTTGGTGAT GCGTAAGACA TTGCAGTGGG AAACCATTCA ACTTGAGTTT 660

ATTGGAGTTT GCTGTTGTAG CAGGTTTTAA CTCAGGAACA ACTCTTGTCT GATCTCTCGC 720

CCCTCTGCCG GGACTACATT ACTGTCTCTC GGAGCCGGTA GCGTTGCTGT CGAGTCCCAG 780

GACTATCTCT GCAGACTGCT ATGCTCAGAT CGAAGTATTT CACAAGAATA CTTGTGTTTT 840

TAACAGCCCT TCCCCTGGAC GGTGCGCCAT GAGGGCCTCA TGTTACGCAT TGCCTTTTCT 900

TTCTGTGGAT CCAGTATCTT CCTCGGCTTT TTAGGGAGCA GGAAAAATGC GTCTGAGAGC 960

AACTCTTTTT AAAAACCTGC CCTGTTGTAT ATAACTGTGT CTGTTTCACC GTGTGACCTC 1020

CAAGGGGGTG GGAACTTGAT ATAAACGTTT AAAGGGGCCA CGATTTGCCC GAGGGTTACT 1080

CCTTTGCTCT CACCTTGTAT GGATGAGGAG ATGAAGCCAT TTCTTATCCT GTAGATGTGA 1140

AGCACTTTCA GTTTTCAGCG ATGTTGGAAT GTAGCATCAG AAGCTCGTTC CTTCACACTC 1200

AGTGGCGTCT GTGCTTGTCC ACATGCGCTG GGCGTCTGGA CCTTGAATGC CTGCCCTGGT 1260

TGTGTGGACT CCTTAATGCC AATCATTTCT TCACTTCTCT GGACACCCAG GGCGCCTGTT 1320

GACAAGTGTG GAGAAACTCC TAATTTAAAT GTCACAGACA ATGTCCTAGT GTTGACTACT 1380

ACAATGTTGA TGCTACACTG TTGTAATTAT TAAACTGATT ATTTTTCTTA TGTCAAAAAA 1440

AAA 1443

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 213 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GATCCCGTTT GACAGGTGTA CCGCCCCAGT CAAACTCCCC ACCTGGCACT GTCCCCGGAG 60

CGGTCGCGCC CGCGCGACCA CGGAGCTCTG GGCGCCAGAA GCGAGAGCCC CTCGCTGCCC 120

CCCGCCTCAC CGGGTAGTGA AAAAACGATG AGAGTAGTGG TATTTCACCG GCGGCCCGCG 180

AGGACCCCCG CCCGACCCAG TGCGGAACGG GGG 213

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 358 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CACCGTGGAT TACTGGTCTC AGAACAACTC ATTGCGCATC AGATTTACTC TCTGANTTTC 60

TGTCTATTGG CCATTGCCCT TTAACTGCAC CTGAATCCTT TGTGTACTGA TCCTTTGAGC 120

TGGGCACCTT GGGAGAGTGT TGTGTTGCTG TTTACGGTTC TTCCTTCCCC TTGCTAATTA 180

CAGTCTCTGG TGCCAGCAAG CCCCTTTGGC TTCCTTCCGT GACTGGTCAC GTTGTCTGCC 240

TGGGCCAGCG TGGCCCCATG CTGCAGAACC TGGCCTCAGG ACTTTTCACT AGAATTGCCC 300

TTCCTCAACT TAGCAGATCA TTCACTCATG CGGGCACAAG CAAAGATCAA CACTTTCT 358

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 378 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CATTGCAGCT CAGCATGGCT CAGACCAGCT CATACTTCAT GCTGATCTCC TGCCTGATGT 60

TTCTGTCTCA GAGCCAAGGC CAAGAGGCCC AGACAGAGTT GCCCCAGGCC CGGATCAGCT 120

GCCCAGAAGG CACCAATGCC TATCGCTCCT ACTGCTACTA CTTTAATGAA GACCGTGAGA 180

CCTGGGTTGA TGCAGATCTC TATTGCCAGA ACATGAATTC GGGCAACCTG GTGTCTGTGC 240

TNCCCAGGCC GAGGGTGCCT TTGTGGCCTC ACTGATTAAG GAGAGTGGCA CTGATGACTT 300

CAATGTCTGG ATTGGCCTCC ATGACCCCAA AAAGAACCGC CGCTGGCACT GGAGCAGTGG 360

GTCCCTGGTC TCCTACAA 378

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 142 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TTTTCCTAGA ACAAGGGTTC TATCTTTAAA AACCCAAATT GCAGCACCGC TGGTCTCAGA 60

ACAACTCATT GCGCATCAGA TTTACTCTCT GATTTTTCTG TCTATTTGGC CAAATTGCCC 120

TTTTAACTGC ACCTGAATCT TT 142

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 151 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TTCTCCTATA GAGATTGTTG ATTTGCCTCT TAGCAAGAGA TTCATTGCAG CTCAGCATGG 60

CTCAGACCAG CTCATACTTC ATGCTGATCT CCTGCCTGAT GTTTCTGTCT CAGAGCCAAG 120

GTAAGATCTC TTTTCCACCA ACCAACTCTT T 151

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CACCGTGGAT TACTGGTCTC AGAACAACTC ATTGCGCATC AGATTTACTC TCTGATTTTT 60

CTGTCTATTG GCCAA 75

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CATTGCAGCT CAGCATGGCT CAGACCAGCT CATACTTCAT GCTGATCTCC TGCCTGATGT 60

TTCTGTCTCA GAGCCAA 77

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 130 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GAATTCCTGG GCTCAAGTGA TCCTCTCATG CAGTCTCCCA AAGTGCTGGG ATGACAGGCT 60

TGAGCCACCA CACCAGGCCC ATCATCAGTT TATATAAAGA AAAAAAAACC TTAAAATTGT 120

TAGGCAAATA 130

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 144 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGAGTTTTGT CATCAGGCCA GCCTCATCCC GAGGTCTCCT CCACCATTGG CCGTAGCCAG 60

CAGGTTCAGT GCTCACCGAA AGTAAAATCC CCTCCTTCAG CAAGAATAAA GCAATATACA 120

CCTTAGGTTC CACTAAGTAA CATA 144

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTTCTGTGAG TCTCAATTTG TTCCTTCTTG GAAGCTGTCT GGTGAATCTG TTGGTCCCTC 60

TGTCTGCTAT TCTGTCTGTC TGTATGTCTG TCCATG 96

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 105 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GTTGATTTGC CTCTTAAGCA AGAGATTCAT TGCAGCTCAG CATGGCTCAG ACCAGCTCAT 60

ACTTCATGCT GATCTCCTGC CTGATGTTTC TGTCTCAGAG CCAAG 105

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 215 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both

(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ACTACCAAAC CTGCATTAAA AAATTTCGGT TGGTCGACCT CGGAGCAGAA CCCAACCTCC 60

GAGCAGTACA TGCTAAGACT TCACCAGTCA AAGCGAACGT ACTATACTCA ATTGATCCAA 120

TAACTTGACC AACGGAACAA GTTACCCTAT AACAGCGCAA TCCTATTCTA GAGTCCATAT 180

CAACAGGGTT TACGACCTCG ATGTTGGATC AGGAC 215

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACCACGGAGC TCTGGGCGCC AGAAGCGAGA GCCCCTCGCT GCCCCCCGCC TCACCGGGTA 60

GTGAAAAAAC GATGAGAGTA GTGGTATTTC ACCGGCGGCC CGCGAGGACC CCCGCCCGAC 120

CCAGTGCGGA ACGGGGGAGT AGTCCCGGGG GCTCACTTAT TCTACATTAG TCTCACGTGC 180

AGACTAGAGT CAAGCTCAAC AGGGTCTTCT TTCCCGCTGA TTCCGCCAAG TC 232

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AGTTTCACTC TGTTGCCCAG GCTGGAGTGC AATGGCACAA TCCTGGCTCA CTGCAACCTC 60

CGCCTCCCGA GCTCAAGCAA TTCTCCTGCC TCAGCCTCGT GAGCCGCTGG GA 112

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AGATCTCGCT CTGTCACCCA GGCTGAAGTG CAGTGGCCCA ATCTCGGCTC ACTGCGAGCT 60

CCACCTCCCG GGTTCACTTC ATTCTCCTGC CTCACTGCCT CAGCCTCTGA GTAGCTGGGA 120

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 594 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GAGGCGTATT ATACCATGCT CCATCTGCCT ACGACAAACA GACCTAAAAT CGCTCATTGC 60

ATACTCTTCA ATCAGCCACA TAGCCCTCGT AGTAACAGCC ATTCTCATCC AAACCCCCTG 120

AAGCTTCACC GGCGCAGTCA TTCTCATAAT CGCCCACGGG CTTACATCCT CATTACTATT 180

CTGCCTAGCA AACTCAAACT ACGAACGCAC TCACAGTCGC ATCATAATCC TCTCTCAAGG 240

ACTTCAAACT CTACTCCCAC TAATAGCTTT TTGATGACTT CTAGCAAGCC TCGCTAACCT 300

CGCCTTACCC CCCACTATTA ACCTACTGGG AGAACTCTCT GTGCTAGTAA CCACGTTCTC 360

CTGATCAAAT ATCACTCTCC TACTTACAGG ACTCAACATA CTAGTCACAG CCCTATACTC 420

CCTCTACATA TTTACCACAA CACAATGGGG CTCACTCACC CACCACATTA ACAACATAAA 480

ACCCTCATTC ACACGAGAAA ACACCCTCAT GTTCATACAC CTATCCCCCA TTCTCCTCCT 540

ATCCCTCAAC CCCGACATCA TTACCGGGTT TTCCTCTTAA AAAAAAAAAA AAAA 594

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Glu Ala Tyr Tyr Thr Met Leu His Leu Pro Thr Thr Asn Arg Pro Lys
1 5 10 15

Ile Ala His Cys Ile Leu Phe Asn Gln Pro His Ser Pro Arg Ser Asn
20 25 30

Ser His Ser His Pro Asn Pro Leu Lys Leu His Arg Arg Ser His Ser
35 40 45

His Asn Arg Pro Arg Ala Tyr Ile Leu Ile Thr Ile Leu Pro Ser Lys
50 55 60

Leu Lys Leu Arg Thr His Ser Gln Ser His His Asn Pro Leu Ser Arg
65 70 75 80

Thr Ser Asn Ser Thr Pro Thr Asn Ser Phe Leu Met Thr Ser Ser Lys
85 90 95

Pro Arg

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 554 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

ATACCATGCT CCATCTGCCT ACGACAAACA GACCTAAAAT CGCTCATTGC ATACTCTTCA 60

ATCAGCACAT AGCCCTCGTA GTAACAGCCA TTCTCATCCA AACCCCCTGA AGCTTCACCG 120

GCGCAGTCAT TCTCATAATC GCCCACGGGC TTACATCCTC ATTACTATTC TGCCANCAAA 180

CTCAAACTAC GAACGCACTC ACAGTCGCAT CATAATCTCT CTCAAGGACT TCAAACTCTA 240

CTCCCAAGCT TTGTGACTTC TAGCAACCTC GCTAACCTCG CCTTACCCCC ACTATTAACT 300

ACTGGGAGAA TGTGCTAGTA ACCACGTTCT CCTTCAAATA TCACTCTCCT ACTTACAGGA 360

CTCAACATAC TAGTCCAGCC CTATACTCCC TCTACATATT TACCACAACA CAATGGGCTC 420

ACTCACCCAC CACATTAACC ATAAAACCCT CATTCACACG AGAAAACACC CTCATGTTCA 480

TACACCTATC CCCCATTCTT CCTATCCCTC AACCCCGACA TCAACCGGGT TTCCTCTTAA 540

AAAAAAAAAA AAAA 554

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 590 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ATGCCTATCG CTCCTACTGC TACTACTTTA ATGAAGACCG TGAGACCTGG GTTGATGCAG 60

ATCTCTATTG CCAGAACATG AATTCGGGCA ACCTGGTGTC TGTGCTCACC CAGGCCGAGG 120

GTGCCTTTGT GGCCTCACTG ATTAAGGAGA GTGGCACTGA TGACTTCAAT GTCTGGATTG 180

GCCTCCATGA CCCCAAAAAG AACCGCCGCT GGCACTGGAG CAGTGGGTCC CTGGTCTCCT 240

ACAAGTCCTG GGGCATTGGA GCCCCAAGCA GTGTTAATCC TGGCTACTGT GTGAGCCTGA 300

CCTCAAGCAC AGGATTCCAG AAATGGAAGG ATGTGCCTTG TGAAGACAAG TTCTCCTTTG 360

TCTGCAAGTT CAAAAACTAG AGGCAGCTGG AAAATACATG TCTAGAACTG ATCCAGCAAT 420

TACAACGGAG TCAAAAATTA AACCGGACCA TCTCTCCAAC TCAACTCAAC CTGGACACTC 480

TCTTCTCTGC TGAGTTTGCC TTGTTAATCT TCAATAGTTT TACCTACCCC AGTCTTTGGA 540

ACCTTAAATA ATAAAAATAA ACATGTTTCC ACTAAAAAAA AAAAAAAAAA 590

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 466 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CTTCTTTTTC AGGCCAAGAG GCCCAGACAG AGTTGCCCCA GGCCCGGATC AGCTGCCCAG 60

AAGGCACCAA CCTATCGCTC CTACTGCTAC TACTTTAATG AAGACCGCGA GACCTGGGTT 120

GATGCAGTGT GAGTGAGGAG AGCGTGTGGG AAGGGAGACT CATGAAGGGA GGGGAAGCTG 180

CCACTCTCCA GTGTTCAGTG GCGCAATGAG ATGAGACTGA ACCCCTTTAT ACTATCATCA 240

GCCCCAAACT TTCCAATCTA CTTTATCCCA TTATTCAGCA CATTCCCAGC ACAAAGAACC 300

TGGTGGGTGA CAGCATCATC ACGGACATTA CTCTGCTGTC CTTTTTCACC CTCCTCTTGG 360

AGGACTCAGT ATATCCGTCA CAACCCTCCA CTGAGTCTCC ATTTTCTTCT GCAACAGCTC 420

TATTGCCAGA ACATGAATTC GGGCAACCTG GTGTCTGTGC TCACCC 466

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGTATTATAC CATGCTCCAT CTGCCTACGA CAAACAGACC TAAAATCGCT CATTGCATAC 60

TCTTCAATCA GCCACATAGC CCTCGTAGTA ACAGCCATTC TCATCCAAAC CCCCTGAAGC 120

TTNCCGGCGC AGTCATTCTC ATAATCGCCC ACGGGCTTAC ATCCTCATTA CTATTCTGCC 180

TAGCAAACTC AAACTACGAA CGCACTCACA GTCGCATCAT AATCCTCTCT CAAGGACTTC 240

AAACTCTACT CCCACTAATA GCTTTTTGAT GACTTCTAGC AAGCCTCGCT AACCTCGCCT 300

TACCCCCCAC TATTAACCTA CTGGGAGAAC TCTCTGTGCT AGTAACCACG TTCTCCTGAT 360

CAAATATCAC TCTCCTACTT ACAGGACTCA ACATACTAGT CACAGCCCTA TACTCCCTCT 420

ACATATTTAC CACAACACAA TGGGGCTCAC TCACCCACCA CATTAACAAC ATAAAACCCT 480

CATTCACACG AGAAAACACC C 501

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 372 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GTTCTTAGTC TATCTCTTGT ACAAACGATG TGCTTTGAAG ATGTTAGTGT ATAACAATTG 60

ATGTTTGTTT TCTGTTTGAT TTTAAACAGA GAAAAAATAA AAGGGGGTAA TAGCTCCTTT 120

TTTCTTCTTT CTTTTTTTTT TTCATTTCAA AATTGCTGCC AGTGTTTTCA ATGTAGGACA 180

ACAGAGGGAT ATGCTGTAGA GTGTTTTTAT TGCCTAGTTG ACAAAGCTGC TTTTGAATGC 240

TGGTGGTTCT ATTCCTTTGC ACATCACGAC ATTTTATAAT CATAGTTAAA TCGTATATGA 300

CAAAAATGCT CTGATCTGAT GCCAAAGGTC AATTCAGTGT ATATAACCTG AACACACTCA 360

TCCATTGCGT TT 372

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Phe Val Phe Cys Leu Ile Leu Asn Arg Glu Lys Ile Lys Gly Gly
1 5 10 15

Asn Ser Ser Phe Phe Leu Leu Ser Phe Phe Phe Ser Phe Gln Asn Cys
20 25 30

Cys Gln Cys Phe Gln Cys Arg Thr Thr Glu Gly Tyr Ala Val Glu Cys
35 40 45

Phe Tyr Cys Leu Val Asp Lys Ala Ala Phe Glu Cys Trp Trp Phe Tyr
50 55 60

Ser Phe Asp Thr
65

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 377 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ACTGTCTCCC CCTTTGATAG GGACACTAAA GTGGTCTGTA CTTGGGTAGA GGATGGCANG 60

TTAAGAATTA AAATCGTCTG GGTGCGGTCT GCACGCTTGT AATCCCAGCA CTTTGGGAGG 120

CTGAGGCGGG CGGATCACCT GAGGTCAGGA GTTCGACACC AGCCTGATGA ACATGGAGAA 180

ACCCCATCTC TACTAAAAAT ACAAATATTA GCTGGGCGTT GTCGCGCGCC TGTAATCCCA 240

GCGGCTCACG AGGCTGAGGC AGGAGAATTG CTTGAGCTCG GGATGGCGGA GGTTGCAGTG 300

AGCCAGGATT GTGCCATTGC ACTCCAGCCT GGGCAACAAG AGTGAAACTC TGTCTCAAAA 360

AAAAAAAAAA AAAAAAA 377

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1480 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GCGTAAACAC ATTTTTGTTC TTAGTCTATC TCTTGTACAA ACGATGTGCT TTGAAGATGT 60

TAGTGTATAA CAATTGATGT TTGTTTTCTG TTTGATTTTA AACAGAGAAA AAATAAAAGG 120

GGGTAATAGC TCCTTTTTTC TTCTTTCTTT GATTTTAAAC AGAGAAAAAA TAAAAGGGGG 180

TAATAGCTCC TTTTTTCTTC TTTCTTTTTT TTTTTTCATT TCAAAATTGC TGCCAGTGTT 240

TTCAATGATG GACAACAGAG GGATATGCTG TAGAGTGTTT TATTGCCTAG TTGACAAAGC 300

TGCTTTGAAT GCTGGTGGTT CTATTCCTTT GACACTACGC ACTTTTATAA TACATGTTAA 360

TGCTATAGGA CAAGATGCTC TGATTCCTGA GTGCCAGAGG TTCAATTCAG TGTATATAAC 420

TGAACACACT CATCCATTTG TGCTTTTGTT TTTTTTATGG TGGCTTAAAG GTAAAGAGCC 480

CATCCTTTGC AAGTCATCCA TGTTGTTACT TAGGCATTTT ATCTTGGCTC AAATTGTTGG 540

AAGAATGGTG GCTTGTTTCA TGGTTTTTGT ATTTGTGTCT AATGCACGTT TTAACATGAT 600

AGACGCAATG CATTGTGTAG CTAGTTTTCT GGAAAAGTCA ACTCTTTTAG GAATTGTTTT 660

TCAGATCTTC AATAAATTTT TTCTTTAAAT TTCAAAGAAC AATGTGCTTG TGTTGATGCC 720

TTACAAAAAC CATTGTATAT TTGTGTATTC CTTCTTGTAT TTAGACAGTG GTTTTTCAGG 780

TGCGTGCTTT GTTTTCTGGT ATGGCCTTTA TGGAATGAGA CGCTTTAGCT TTGGTACGTA 840

GCGCTAATCC ATAGCAGCTT TGGCAGTTTC GTGTCTTGAG TCTTAGCTAA AAAGTTAGAA 900

GTTTACATGA CTGTTTTTTT TATTTTCCCT AAATTATTAC TTACTCTGAG CATTAATTAA 960

GGGCATTTTC ACCTGTGTAA AATTATGGTC AGCTTTTTTC TGTCTATAAT TGTTTACTTT 1020

TGTGGGTTTA CTCTAGAAAC ATGAGCCAAA AATGTCAATA GACAACACAG TATTAAAATA 1080

ACCCAAAAGT TGTAAAGGGC AACGTTTCTC CCCTTTGATA GGGACACTAA AGTGGTCTGT 1140

ACTTGGGTAG AGGATGGCAG ACGTTAAGAA TTAAAATGCG TCTGGGTGCG GTCTCACGCT 1200

TGTAATCCCA GCACTTTGGG AGGCTGAGGC GGGCGGATCA CCTGAGGTCA GGAGTTCGAC 1260

ACCAGCCTGA TGAACATGGA GAAACCCCAT CTCTACTAAA AATACAAATA TTAGCTGGGC 1320

GTTGTCGCGC GCCTGTAATC CCAGCGGCTC ACGAGGCTGA GGCAGGAGAA TTGCTTGAGC 1380

TCGGGATGGC GGAGGTTGCA GTGAGCCAGG ATTGTGCCAT TGCACTCCAG CCTGGGCAAC 1440

AAGAGTGAAA CTCTGTCTCA AAAAAAAAAA AAAAAAAAAA 1480

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 381 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

TTGGGTGAGG TGGAATGATG GTCTGTTGGA ATCGGTTTGG TAAATGGGTT TATTTCATAT 60

CCGCTATCTT TAACTTTGGA CCGCGTTATC TATATCATGG CGTTCCTTTC TACTTTTTAA 120

TATTGGTTCG TATTATATCG TTCCTGATTG GGGATATGGA AGACGTATTA CTTAATTGTA 180

CTTTATTGAA ACGTTCCTCT CGGTTTCGAT TCTGGGGGCT TTGGTCTGCT CGATGGATTC 240

TTGTCGATTT TCTCGTGTGG CAGTAACATA CCGTTTTATC ACCCTTCTAA ATATCCCATC 300

TCCCGCTGTT TGGTAGGCTC GGAACACTAT CGACCAACAG GTTCTATCTA GAATCAAGTT 360

GGAAATTAAA CGGTGTCTTG G 381

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Met Val Cys Trp Asn Arg Phe Gly Lys Trp Val Tyr Phe Ile Ser
1 5 10 15

Ala Ile Phe Asn Phe Gly Pro Arg Tyr Leu Tyr His Gly Val Pro Phe
20 25 30

Tyr Phe Leu Ile Leu Val Arg Ile Ile Ser Phe Leu Ile Gly Asp Met
35 40 45

Glu Asp Val Leu Leu Asn Cys Thr Leu Leu Lys Arg Ser Ser Arg Phe
50 55 60

Arg Phe Trp Gly Ala Leu Val Cys Ser Met Asp Ser Cys Arg Phe Ser
65 70 75 80

Arg Val Ala Val Thr Tyr Arg Phe Ile Thr Leu Leu Asn Ile Pro Ser
85 90 95

Pro Ala Val Trp Met Ala Arg Asn Thr Ile Asp Gln Gln Val Leu Ser
100 105 110

Arg Ile Lys Leu Glu Ile Lys Arg Cys Leu
115 120

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 420 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CCCACAGGTC CTAAACTACC AAACCTGCAT TAAAAAATTT CGGTTGGTCG ACCTCGGAGC 60

AGAACCCAAC CTCCGAGCAG TACATGCTAA GACTTCACCA GTCAAAGCGA ACGTACTATA 120

CTCAATTGAT CCAATAACTT GACCAACGGA ACAAGTTACC CTAGGGATAA CAGCGCAATC 180

CGATGGTGCA GCCGCTATTA AAGGTTCGTT TGTTCAAACG ATTAAAGTCC TCGTGTCTGA 240

GTTCAGACCG AAGTAATCCA GGTCGGTTTC TATCTTCTTC AAATTCCTCC CTGTACCGAA 300

AGGACTAATG AGAAATAAGG CCTACTTCAC AAAGCGGCCT TCCCCCGTAA TGATATCATC 360

TCAACTTAGT ATTATACCCA CACCCACCCA AGAACAGGTT TGTTAAAAAA AAAAAAAAAA 420

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 381 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

AACCCACTCC ACCTTACTAC CAGACAACCT TAGCCAAACC ATTTACCCAA ATAAAGTATA 60

GGCGATAGAA ATTGAAACCT GGCGCAATAG ATATAGTACC GCAAGGAAAG ATGAAAAATT 120

ATAACCAAGC ATAATATAGC AAGGACTAAC CCCTATACCT TCTGCATAAT GAATTAACAT 180

GAAATAACTT TGCAAGGAGA GCCAAAGCTA AGACCCCCGA AACCAGACGA GCTACCTAAG 240

AACAGCTAAA AGAGCACACC GTCATTGTAT GGCAAAATAG TGGGAAGATT TATAGGGTAG 300

AGGGCGACAA ACCATCCGAG CCTTGTGATA GCTGGTTGTC CAAGATAGAT CTTAGTTCAA 360

CCTTTAATTT GCCACAGAAC C 381

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 629 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TTTTTTTTTT TTTTTAACAA ACCCTGTTCT TGGGTGGGTG TGGGTATAAT ACTAAGTTGA 60

GATGATATCA TTACGGGGGA AGGCCGCTTT GTGAAGTAGG CCTTATTTCT CATTAGTCCT 120

TTCGGTACAG GGAGGAATTT GAAGAAGATA GAAACCGACC TGGATTACTT CGGTCTGAAC 180

TCAGACACGA GGACTTTAAT CGTTTGAACA AACGAACCTT TAATAGCGGC TGCACCATCG 240

GGATGTCCTG ATCCAACATC GAGGTCGTAA ACCCTATTGT TGATATGGAC TCTAGAATAG 300

GATTGCGCTG TTATCCCTAG GGTAACTTGT TCCGTTGGTC AAGTTATTGG ATCAATTGAG 360

TTTAGTAGTC CGCTTGGAGT GGTGAAGTCT AGAATGTCCT GTTCGGGGGT TGGTTTCTGC 420

TCCCAGGTCG CCCCAACCGA ATTTTTTATT GAAGGTTGGG TAGTTTAGCA CCTGTGGGTT 480

GGTAAGGTAC TGTTGGAATT AATAAATTAA AGCTCCATAG GGTCTCCTCG TCTTGTTGTG 540

TAATGCCCCC CTCTCCACGG GAAGGTCAAT TCCACTGGTT AAAAGTAAGA GAAAGCTGAA 600

CCCTCGGGGA GCCATCCATA CAGGTCCCC 629

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 256 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GCGGGTAAAT TGGTTTGTTA TTTTTTAAAA AAAACTTGCA TGTTTAAAAA AAAGTTGATT 60

GCTTCAAATT TCTGCTACTA ACTTCAAGCT ATGGGAGTTT GGCAGTAGTC ACTTGAGGAT 120

TTTTTTTCCA ATTCTTTTCT TTTTGTTGTT AAAGCTGTAC TTCAGTGAAC AGAAAAATTG 180

CCAAGCAAAC TAATGGACTA TAAAGCGTAA TTTGACTGTG TGGGACTAAA CTACAGAGCC 240

TACTTGACCA GTGGAT 256

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 270 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CATGTTTAAA AAAAAGTTGA TTGCTTCAAA TTACTGCTAC TAACTTCAAG CTATGGGAGT 60

TTGGCAGTAG TCACTTGAGG ATTTTTTTTC CAATTCGTTT TCATTTTTGT TGTTAAAGCT 120

CGTACTTCAG TGAGACAGAA AAATTGCCAA GCTAAACTAA TGGTCTATAA AAGCGTAATT 180

TGCATGTGTG GGCAAAAACT ACAGAGCCTC AATTGCCACT GAGGTATAGT ACAAAGTTTT 240

AATACATTTT GTAAATCAAA TTGAAAGAAA 270

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 270 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CATGTTTAAA AAAAAGTTGA TTGCTTCAAA TTACTGCTAC TAACTTCAAG CTATGGGAGT 60

TTGGCAGTAG TCACTTGAGG ATTTTTTTTC CAATTCGTTT TCATTTTTGT TGTTAAAGCT 120

CGTACTTCAG TGAGACAGAA AAATTGCCAA GCTAAACTAA TGGTCTATAA AAGCGTAATT 180

TGCATGTGTG GGCAAAAACT ACAGAGCCTC AATTGCCACT GAGGTATAGT ACAAAGTTTT 240

AATACATTTT GTAAATCAAA TTGAAAGAAA 270

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 184 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TCTGCCCAGG CTGGTCTGAA ATTCCTGGGC TGAAGTGATC CTCCAGTCTT GGCCTCCCAA 60

AGTGCTGGGA TTACAGGCAT GAGCTACTGA GCCTAGCCTT AATGATTAAT TTTAGAGTGA 120

TGGCTTGTAC CTTCAAGACA CATATAGATT GAGACAGAAA ATTTCCATCG TCCCCGAGAA 180

AACT 184

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Ser Ser Ser Leu Gly Leu Pro Lys Cys Trp Asp Tyr Arg His Glu Leu
1 5 10 15

Leu Ser Leu Ala Leu Met Ile Asn Phe Arg Val Met Ala Cys Thr Phe
20 25 30

Lys Gln His Ile Glu Leu Arg Gln Lys Ile Ser Ile Val Pro Arg Lys
35 40 45

Leu Cys Cys Met Gly Pro Val Cys Pro Val Lys Ile Ala Leu Leu Thr
50 55 60

Ile Asn Gly His Cys Thr Trp Leu Pro Ala Ser
65 70 75

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1381 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TTTTTTTTTT GAGATGGAGT TTTCGCTCTT GTTGCCCAGG CTGGAGTGCA ATGGCGCAAT 60

CTCAGCTCAC CGCAACCTCC GCCTCCCGGG TTCAAGCGAT TCTCCTGCCT CAGCCTCCCC 120

AGTAGCTGGG ATTACAGGCA TGTGCACCAC GCTCGGCTAA TTTTGTATTT TTTTTTAGTA 180

GAGATGGAGT TTAACTCCAT GTTGGTCAGG CTGGTCTCGA ACTCCCGACC TCAGATGATC 240

TCCCGTCTCG GCCTGCCCAA AGTGCTGAGA TTACAGGCAT GAGCCACCAT GCCCGGCCTC 300

TGCCTGGCTA ATTTTTGTGG TAGAAACAGG GTTTCACTGA TGTTGCCCAA GCTGGTCTCC 360

TGAGCTCAAG CAGTCCACCT GCCTCAGCCT CCCAAAGTGC TGGGATTACA GGCGTCAGCC 420

GTGCCTGGCC TTTTTATTTT ATTTTTTTTA AGACACAGGT GTACCACTCT TACCCAGGAT 480

GAAGTGCAGT GGTGTGATCA CAGCTCACTG CAGCCTTCAA CTCCTGAGAT CAAGCAATCC 540

TCCTGCCTCA GCCTCCCAAG TAGCTGGGAC CAAAGACATG CACCACTACA CCTGGTAATT 600

TTTATTTTTA TTTTTAATTT TTTGAGACAG AGTCTCACTC TGTCACCCAG GCTGGAGTGC 660

AGTGGCGCAA TCTTGGCTCA CTGCAACCTC TGCCTCCCGG GTTCAAGTTA TTCTCCTGCC 720

CCAGCCTCCT GAGTAGCTGG GACTACAGGC GCCCACCACG CCTAGCTAAT TTTTTTGTAT 780

TTTTAGTAGA GATGGGGTTT CACCATGTTC GCCAGGTTGA TCTTGATCTC TTGACCTTGT 840

GATCTGCCTG CCTCGGCCTA CCCAAAGTGC TGGGATTACA GGTCGTGACT CCACGCCGGC 900

CTATTTTTAA TTTTTGTTTG TTTGAAATGG AATCTCACTC TGTTACCCAG GTCGGAGTGC 960

AATGGCAAAT CTCGGCTACT CGCAACCTCT GCCTCCCGGG TCAAGCGATT CTCCTGTCTC 1020

AGCCTCCCAA GCAGCTGGGA TTACGGGACC TGCACCACAC CCCGCTAATT TTTGTATTTT 1080

CATTAGAGGC GGGTTTACCA TATTTGTCAG GCTGGGTCTC AAACTCCTGA CCTCAGGTGA 1140

CCCACCTGCC TCAGCCTTCC AAAGTGCTGG GATTACAGGC GTGAGCCACC TCACCCAGCC 1200

GGCTAATTTG GAATAAAAAA TATGTAGCAA TGGGGGTCTG CTATGTTGCC CAGGCTGGTC 1260

TCAAACTTCT GGCTTCAGTC AATCCTTCCA AATGAGCCAC AACACCCAGC CAGTCACATT 1320

TTTTAAACAG TTACATCTTT ATTTTAGTAT ACTAGAAAGT AATACAATAA ACATGTCAAA 1380

C 1381

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2520 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CCATTGTTAG GTTGTCTCTT ACCTGTTAAA ATCAGGAGCT GACAAGAAAT GCTTACCACA 60

AAAGGAGAAA TGCCAGTCTA GTTAACAGTC AAGGAGAGAA ATCAGGAAGA TTATGTGGGT 120

GGAAGAAGTA GATGATGTGG CTGATGAGTG AGTGAGTGAG CAAGCCTCCG CCCAGCTGAA 180

GAAGGAGTCA GAACTGCCCT TTGTTCCCAA CTATTTGGCG AACCCCAGCC TTCCCTTTTA 240

TCTATACACC CACAGCAGAG GATTCAGCCC AGATGCAGAA TGGGGGCCCC TCCACACCCC 300

CTGCATCACC CCCTGCAGAT GGCTCACCTC CATTGCTTCC CCCTGGGAAC CTCCCCTGTT 360

AGGGACCTTT CCCCGGGACC ACACCTCTTT GGCACTAGTT CAGAATGGTG ATGTGTCGGC 420

CCCTCTGCCA TACTAGAACA CCAGAAAGAC AAACGGGTGA TGTTTGTCAG CTACAGTGAG 480

TCTAGAGCCG TCCTGTTTTC TTCTGTCCCG TCCCAAGCCA CCATGTCTCT TCGAGCCTCA 540

AAATGGGACG TATGCAGGAC CAGCGCCCAG ATTCCAAGCC ATTTTTCTTC ACTGGAGCAT 600

TTCCATTTAA TATGCAAGAG CTGGTACTCA AGGTGAGAAT TCAGAACCCA TCTCTTCGAG 660

AAAATGATTT CATTGAAATT GAACTGGACC GACAGAGCTC ACCTACCAAG AGTTGCTCAG 720

AGTGTGTTGC TGTGAGCTGG GTGTTAATCC AGATCAAGTG GAGAAGATCA GAAAGTTACC 780

CAATACTCTG TTAAGGAAGG ACAAGGATGT TGCTCGACTC AAGATTTCAG GAGCTGGAAC 840

TGGTTCTGAT GATAGTGAAA ATAATTTTCT GTTCAGAAAT GCTGCATCAC ACTGACTGAA 900

AGGCCTTGCT ATACAGGAGA GCTTCAAAAC TGACTTACTA ATGCAGCAGG GACTTTTATA 960

CTGAGTATAT GACAGTGTGC ATCACCTCTG GGCCAAGGAC AAGCCATGAT CTAAATGCCT 1020

CAGATGCCCG GGCCAGTCTG GTGCACTGCA TAGTATATAC GAACATCATT CTGCCCAAGG 1080

TAGGAAGCCC CATGACCCCC AAGCAGTGGT GTCCACTCTT CCAAGCCTCT TGGTGCACAA 1140

TAAACCTTAT TGCTTGAAGC TTTGAACGAC TGTGAGAATG GTCTGGCGAG GACGAGAACG 1200

TGGAATTATA TGAGTGTCTT TTGTATCCGA GAATGTAGAG AGTTCTCTGA AGACGACGAC 1260

TGAGAGAGAG CGGACGCTAT TTCTAGCCAC TCCTGTTGAC AGTGCACCTG AAGGGCTGGG 1320

ATGCGTTTTT CTTGGTGTTG CATGCTCACA ACTCTGCTGA CATTGGGAAC TTATGAGAGA 1380

GGAAGACTCG GGAAAGCACA GATACTGGAC AGATGGATTC TGGTGTGGGG AAAGCACAGA 1440

TACTGGACAG ATGGTTCTAG TGTGACTTGT GACTGTGAGG TTTCCTATAA CATATTTATA 1500

AATGTTCATC AGGTTCAAAA GTCTATAAGA ATACAGTTCG AGACTGAATT GCTTCGAAAT 1560

ACTTCGATGT TGGGAACCAA AAGAGCTTTC CCTCCCTCAC TTTTTCCTTT GTAACACTCA 1620

TGACTGCTTC TCTGTCTCGA GTCATCTCTG CATTAACTCC CCTTCGTGGT CACTAGAGGG 1680

CTCTCTGATG CTTCTAAGAC ACTGCTTTTT ACATGCCACA CCCACCGCGT AGAGACAGGG 1740

TCTCACTATG TGGCCCAGGC TGGTCTCAAA CTTCTGGCCT TAAGTGATCG TCCTGTCCTT 1800

CGCGCTCGGA AGAAAGTCGT GGGGATTACA GGTGTGAGCC ACCCGCCCAG CCCCTCCCTT 1860

GTGTTTCAAC CAATCGGAAG TGAATTTAAC TAGATGTAGT AACCTTTTTT TTCTTTGACT 1920

TCTAAAAAAG TTACAGTTTA CTAATAAAGT TAAGTCTGGT TCTGTCCTAG AGGAAATAAA 1980

TTCACTATTA ATTCATGTCT TAAGTTACTT GGGTTAAAAC ACTTTCAGCC ACCCAGATTA 2040

ATTAAAGTGG AGCAGTGGAG CCCCTGGCTG GGGAGATGGG CCTCCAGAGG AGCAGCTGCA 2100

GGCATGTTCT GGCTACACAG AGGCAAGCAA GGGACTGGTG TCTCTGGTGA GAGGTGGGTT 2160

TGATGTATCT CTGTCCTATG CTGGTCTCTC TTCTCCTTTA TAAATCCTCC TGTGGTCACT 2220

GACTATCGTA TCGCAGTGAT CAGACTGCAC ATAGTACGGT TAGGCTGAGC TTAATGTCTT 2280

AATCATGTCA TTCGAGAGAA GACACGTTTT GATTCATGCT TTGTGTAATT AATCAATCAA 2340

GGATTCTTTT TTTAGCTTTG TTGACGTGTA ATTCACCCCT CCTCCTCCAC TGCATATTTA 2400

AAGCATGTGT TCACACTGTG TGTATACATT CACTGCGATT TTTTCGTTTG CTGCATTGCT 2460

TGGACTGTTC ATAACATCAC AAGTATTATT CAAATAAAAT ATTAACTGAC CGAAAAAAAA 2520

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GAATTCCTGG GCTCAAGTGA TCCTCTCATG TCAGTCTCCC AAAGTGCTGG GATGACAGGC 60

TTGAGCCACC ACACCAGGCC CATCATCAGT TTTTATATAA AGAAAAAAAA ACCTTAAAAT 120

TGTTAGCAAA ATACTATGAC A 141

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 151 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GAACTCCTGA CCTCAGGTGA TCCGCCCGCC TCAGCCTCCC AAAGTGCTGG GATTACAAGC 60

GTGCAGACCG CACCCAGACG ATTTTAATTC TTAACNTGCC ATCCTCTACC CAAGTACAGA 120

CCACTTTAGT GTCCCTATCA AAGGGGAGAC A 151

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

AATTCTCCTG CCTCAGCCTC GTGAGCCGCT GGGATTACAG GCG 43

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

AAGCCAACTC AGACTCAGCC AACAGGTAAG TGGGCATTAC AGGAG 45

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CTCAGTTTTC ACTTTGATCT GGTAGATAGT TTTCGTTTCA GTTGGGGGAG AAGGATCTGT 60

TTGTAAGAAC GGAGTGACGG GATACCATAA AAATAGAGGT AATAACATAC ATTGGGACGT 120

GTAAATTTAT TTTTATGGAA GTG 143

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 157 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CTCACAGTTT CCTTATCTTG GTGGTCGTAA GTTTTCGTCG AAACAGTTGA TCGTTATTTG 60

TGAGATTGTC GTATAGGGAG ACTAACAGGT AGTAACTTTT GTGACCGTCG TTAAAACTTT 120

ACTTTTTTTT TTCTTTCTTC TTTTTTCCTT CATAATG 157

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ACCACGCCCC GCTAATTTTT GTATTTTTAG TAGAGACAGG GTTTCACCGT GTTGGCCAGG 60

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

ACAACGCCCA GCTAATATTT GTATTTTTAG TAGAGATGGG GTTTCTCCAT GTTCATCAGG 60

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CTGGTCTGAA ATTCCTGGGC TGAAGTGATC CTCCAGTCTT GGCCTCCCAA AGTGCTGGGA 60

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CTGGTGTCGA ACTCCTGACC TCAGGTGATC CGCCCGCCTC AGCCTCCCAA AGTGCTGGGA 60

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

AGACACATAT AGATTGAGAC AGAAAA 26

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

AGTACAGACC ACTTTAGTGT CCCTATCAAA 30

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

AGATCTCGCT CTGTCACCCA GGCTGAAGTG C 31

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

AGAGTTTCAC TCTTGCTTGC CCAGGCTGGA GTGC 34

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

AGTGGCCCAA TCTCGGCTCA CTGCGAGCTC CACCTCCCGG GTTCACTTCA TTCTCCTGC 59

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

AATGGCACAA TCCTGGCTCA CTGCAACCTC CGCCCTCCCG AGCTCAAGAA CTTCTCCTGC 60

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CTCAGCCTCT GAGTAGCTGG GACTACAGGC GCCCACCACA AGCCGCTAAT TTTTGTATTT 60

TTGTAG 66

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CTCAGCCTCG TGAGCCGCTG GGATTACAGG CGCGCGCCAC AAGCGACTAA TATTTGTATT 60

TTTGTAG 67

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

ACCACGCCCC GCTAATTTTT GTATTTTTAG TAGAGACAGG GTTTCACCGT GTTGGCCAGG 60

ATGCTCGATC TCCTGA 76

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

ACAACGCCCA GCTAATATTT GTATTTTTAG TAGAGATGGG GTTTCTCCAT GTTCATCAGG 60

CTGGTGTCGA ACTCCTGA 78

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 159 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

CCCCAAGCAG TGTTAATCCT GGCTACTGTG TGAGCTGACC TCAAGCACAG GTGAAGGCAG 60

AGAATCCATC CACCTGTTTC TGTTCTCCCT GCTTAGCTCC AGGGATGGAA CTGGGACTGG 120

GATAGAGGAA AGGTGAACTC CTCATTAAGG AAATGGATG 159

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 170 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CCCCTGTTCT TGGGTGGGTT TGGGTATATT CTGGTTGAGA TGATATCATT TACGGGGGAA 60

GGCGCTTTGT GAAGTAGGCC TTATTTCTCT TGTCCTTTCG TACAGGGAGG ATTTGAAGTA 120

GTAGAACGCT GTTACTCCGG TCTGAACTCA GTCACGTGGC TTTATCGTTG 170

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GATCCAAGCT ACGTACGCGT GCATGCACGT CATAGCTCTT CTATAGTGTC AC 52

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GATCCGAGCT CGGTACCAAG TTGATGCATA GCTTGAGTAT TCTATAGTGT CAC 53

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GTATGGGCCC GATAGCTTAT TTAGCCTTTA GAGCACACTG GCGGCCGTTA CTAGTGGATC 60

CGAGCTCGGT ACCAACTTGA TGCATAGCTT GAGTATTCTA TAGTGTCACC TAAAT 115

(2) INFORMATION FOR SEQ ID NO:76:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

ATATAGACAA TATAACAATA TATTGTATAC TTTAGAGCAC ACTGGCAGCC GTTACTAGTG 60

GATCCGAGCT CGGTACCAAG TTGATGCATA GCTTGAGTAT TCTATAGTGT CACTAATAGT 120

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

CTTAATAGAT AGCTACTTAA AATAACTTAC ACACTGTTTT AGAGTGCTTG AAAACTATCT 60

GATCAGACAT AGTATTGAAA CCAATGAATA CATTATATAA AGTAAAGGAA AGGAGAA 117

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 137 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

CTTACTACCA GACAACCTTA GCCAAACCAT TTACCCAAAT AAAGTATAGG CGATAGAAAT 60

TGAAACCTGG CGCAATAGAT ATAGTACCGC AAGGAAAGAT GAAAAATTAT AACCAAGCAT 120

AATATAGCAA GGACTAA 137

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 198 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

CTATAAAGGT CGTTGTCAAC GATAAAGCAC GTGACTGAGT TCAGACCGGA GTAACAGCGT 60

TCTACTACTT CAAATCCTCC CTGCGAAAGG CAAGAGAAAT AAGGCCTACT TAAGCGCCTT 120

CCCCCGTAAA TGATATCATC TCAACCAGAA TATACCCAAA CCCCCCAAGA ACAGGGGAGG 180

AAAAGAAAAA AAAAAAAA 198

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 200 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

CTATAGAGAT TGTTGATTTG CCTCTTAAGC AAGAGATTCA TTGCAGCTCA GCATGGCTCA 60

GACCAGCTCA TACTTCATGC TGATCTCCTG CCTGATGTTT CTGTCTCAGA GCCAAGGTAA 120

GATCTCTTTT CCAACTCTTT CTAGCCCTGA AGACTTCACT CTATCCCCAA GCATACGGGT 180

CTACTTGAAA AAAAAAAAAA 200

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 82 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

CAGAAAACTA GCTACACAAT GCATTGGTCT ATCATGTTAA AACGTGCATT AGACACAAAT 60

ACAAAAACCA TGAAACAAGC CA 82

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

CAGAAAAATT GCCAAGCTAA ACTAATGGTC TATAAAAGCG TAATTTGCAT GTGTGGGCAT 60

AAACTACAGA GCTCATGCTA GAGTATGCAA 90

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

AATTGGGTAC CGGGCCCCCC CTAGAGGTCG ACGGTATCGA TAAGCTTGTA TCGAATTCCG 60

GACTTTGCTT TTGGTTTTCC TTTCCTGTGA AAAGGTTGGT TTTAAAGTGA GATACACTTT 120

TCCGTAGAAC AAGTGTTCTA T 141

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

AAGTTGATTG CTTCAAATTT CTGCTACTAA CTTCAAGCTA TGGGAGTTTG GCAGTAGTCA 60

CTTGAGGATT TTTTTTCCAA TTCGTTTTCA TTTTTGTTGT TAAAGCTCGT ACTTCAGTGA 120

GACAGAAAAA TTGCCAAGCT AAACTAATGG TCTAT 155

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

GAATTCCTGG GCTCAAGTGA TCCTCTCATG TCAGTCTCCC AAAGTGCTGG GATGACAGGC 60

TTGAGCCACC ACACCAGCCC ATCATCAGTT TTTATATAAA GAAAAAAAAA CCTTAAAATT 120

GTTAGGCAAA TAATGACAAA TTGTAATATA TATTCTTACA TTTCAGATTT TTATTTTTTA 180

AACTGATAAG AATTGATTAA TAAATAAAAT TTAGTATTAA TCTGTCTTTT AA 232

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 245 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

AAATTCCTGG GCTGAAGTGA TCCTCCAGTC TTGGCCTCCC AAAGTGCTGG GATTACAGGC 60

ATGAGCTACT GAGCCTAGCC TTAATGATTA ATTTTAGAGT GATGGCTTGT ACCTTCAAGC 120

AACATATAGA GTTGAGACAG AAAATTTCCA TCGTCCCGAG AAAACTGTGC TGCATGGGCC 180

CCGTGTGCCC TGTGAAGATC GCCCTATTAA CTATAAATGG GCATTGCACA TGGTTGCCAG 240

CTTCA 245

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 239 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

AACGTTTCGA ACCTATCGTG AAGCCCGATT TTAGAGTTAA TACGGGTGCT TCAAGGGAAC 60

GGGGCTATGA GAAGTTTTCT ACGGGGAGCA TGGAAATTTT CTGTCTCAAT ATGTGCTTGA 120

AGGTACAACC GTATCTAAAA TTAATCATTA AGGCTAGGCT CAGTAGCTCT GCCTGTAATC 180

CCAGCACTTT CGGGAGGCCA AGACTGGAGG ATCACTTCAG CCCAGGAATT TCAGACGCC 239

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

TACTGTGTGA GCCTGACCTC AAGCACAGGA TTCCAGAAAT GGAAGGATGT GCCTTGTGAA 60

GACAAGTTCT CCTTTGTCTG CAAGTTCAAA AACTAGAGGC AGCTGGAAAA TACATGTCTA 120

GAACTGATCC AGCAATTACA ACGGAGTCAA AAATTAAACC GGACCATCTC TCCAACTCAA 180

CTCAACCTGG ACACTCTCTT CTCTGCTGAG TTTGCCTTGT TAATCTTCAA TAGTTTTACC 240

TACCCCAGTC TTTGGAACCT 260

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 149 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

CTTGCTTGCC CAGGCTGGAG TGCAATGGCA CAATCCTGGC TCACTGCAAC CTCCCCCTCC 60

CGAGCTCAAG AACTTCTCCT GCCTCAGCCT CGTGAGCCGC TGGGATTACA GGCGCGCGCC 120

ACAAGCGACT AATATTTGTA TTTTTGTAG 149

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 167 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

CTCGCTCTGT CACCCAGGCT GAAGTGCAGT GGCCCAATCT CGGCTCACTG CGAGCTCCAC 60

CTCCCGGGTT CACTTCATTC TCCTGCCTCA CTGCCTCAGC CTCTGAGTAG CTGGGACTAC 120

AGGCGCCCAC CACCACGTCC CCTGCTAATT TTTTGTATTT TTAGTAG 167

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

CCATGTTCAT CAGGCTGGTG TCGAACTCCT GACCTCGTGA TCCGCCCGCC TCAGCCTCCC 60

AAAGTGCTGG GATTACAAGC GTGC 84

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

CCGTGTTGGC CAGGATGGTC TCGATCTCCT GACCTCGTGA TCCGCCCGCC TTGGCCACCC 60

AAAGAGTTTG GGATTACAGG CGTGC 85

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 251 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

TGCAGCAATG GCAACAACGT CTGCAAACTA TTAACTGGCG AACTACTTAC TCTAGCTTCC 60

CGGCAACAAT TAATAGACTG GATGGAGGCG GATAAAGTTG CAGGACCACT TCTGCGCTCG 120

GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG CCGGTCGAGC GTGGGTCTCG 180

CGTATCATTC GAGCACTGGG GCCAGATGGT AAGCCCTCCG TATCGTAGTT ATCTCACAGC 240

AGGGAGTCAG G 251

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

TGCAGGAGCG GGGAGGCACG ATGGCCGCTT TGGTCCGGAT CTTTGTGAGG AACCTTACTT 60

CTGTGGTGTG ACATAATTGG ACAAACTACC TACAGAGATT TAAAGCTCTA AGGAAATATA 120

AAATTTTTAA GTGTATAATG TGTTAAACTA CTGATTCTAA TTGTTTGTGT ATTTTAGATT 180

CCAACCCTAT GGAACCTGAT GAATGGGAGC CAGTGGTGGA ATGCCTTTAA TGAGGAAACC 240

TG 242

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

TGCAGCAATC TTTCTTATAT ACATGCTTAA TAGATAGCTA CTTGAAATAA CTTACACACG 60

TTTTAGAGTT GCTTGAAAAC TATCTTTCA AGACATAGTA ATTGAAACCA ATGAATACAT 120

TATATAAAGT AAAGGAAAGG AGAAGAGAGG AAAGGGAGGG GAAGAGGAGA GGGAGGGACA 180

AGCGAGAAAG GAAAGGGAAG GGAGAAAA 208

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 152 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

CTCACTAAAG GGATCAAGGA ATAATTTTGA ATTTCAAGTC TTACATTTAA TAAATACATT 60

CATAAGGCTA TAACTACCAT ACGTTGTGAT TTCTCTGATT AATTTAAAAA TAAATTAAAA 120

CCTGGAAAGA ATTTTACCAT TCTAGGAAGC CA 152

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 338 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

AATCTATCTT ATATACATGC TTAATAGATA GCTACTTAAA ATAACTTACA CACGTTTTAG 60

AGTTGCTTGA AAACTATCTG ATCAAGACAT AGTAATTGAA ACCAATGAAT ACATTATATA 120

AAGTAAAGGA AAGGAGAAGA GAGGAAAGGA GGGGAGAGGA GAGGAGGACA AGCGAGAAAA 180

GGAAGGGAAG GGAGAAAAAG GGGGAAAGGG AGGTAGAGAG AGAGAGAAAA AGTGCTGGTC 240

ATATAGTAAG TGTACATTTT AACTTTTTAA GAAACTACCC TACTCTATTC CAGAGTGATT 300

GTACATGTGC ATTTTACTGC ATTATAGAGA TCATTTTC 338

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 169 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

TGCAGGAGTG GGGAGGCACG ATGGCCGCTT TGGTCCGGAT CTTTGTGAAG GAACCTTACT 60

TCTGTGTGTG ACATAATTGG ACAAACTACC TACAGAGATT TAAACGTCTA AGGTAAATAT 120

AAAATTTTTA GTGTATAGGT TAAACTACTG ATTCTAATGT TGTGTATTT 169

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 209 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

CCCCGGGCTG CAGCAATGGC AACAACGTCT GCAAACTATT AACTGGCGAA CTCATTCATC 60

TAGCTTCCCG GCAACAATTA ATGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT 120

CGCGTGGCCC TTCCGGCTGG CTGGTTTATT GCTGATAATT GAGCGTGCGA GTGGCTCGCG 180

TATCATTCGC GACATGGGCC AGTAGGTAC 209

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 272 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

CTTGCCCTTC ATGGAGTCAT ACAGCCGATC AGCAAAATGC AGGGGCTTGT TCTGAATGCA 60

CTGAACCAGG TTCAGGAAAG CATTTTCCAG GTCTCCTTTA ACCTCTTTCC TGATGCTTTC 120

CAACATGTCA TAAGGGCTGT AACTCTTGTA CCTATCAAAT ACTTTCTGGA GGTGGGGACA 180

CGCTCGCGTC GGTCATGATG CTGATCCACT TGGGAACATC AGTTCTTTCC TCTTCACTCC 240

AGCTGCATAG AGATCCGAGG ACTCTTGGTC AA 272

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 278 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

ACGGCCCAGC TTCCTTCAAA ATGTCTACTG TTCACGAAAT CCTGTGCAAG CTCAGCTTGG 60

AGGGTGATCA CTCTACACCC CCAAGTGCAT ATGGGTCTGT CAAAGCCTAT ACTAACTTTG 120

ATGCTGAGCG GGATGCTTTG AACATTGAAA CAGCCATCAA GACCAAAGGT GTGGATGAGG 180

TCACCATTGT CAACATTTTG ACCAACCGCA GCAATGACAC GAGACAGGAT ATTGCCTTCG 240

CCTACCAGAG AAGGACCAAA AAAGGAACTT GCATCACA 278

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 228 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

AGCAATAGCA AAGGAAAGGA AACAATATTT AGCAAGGTTT ATTCTTCCTT TGTGTCAGCA 60

TTTCTGAGTG TGCACACAGG CCCAGTGATT CCATGTATTT TTGAGTGACC ACTGCCTCTG 120

TCTGGCCCTT CCCCATAGAA CCGCCGCTGG TGGAGCGTGG GTCCCTGGTC TCCTACAAGT 180

CCTGGGGCAT TGGAGCCCCA AGCAGTGTTA ATCCTGGCAC TGTGTNAG 228

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 246 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

AGCAATGGCA ACAACGTCTG CAAACTATTA ACTGGCGAAC TACTTACTCT TAGCTTCCGG 60

CAACAATTAA TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC 120

CTTCCGGCTG GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTCGAGCGTG GGTCTCGCGT 180

ATCATTCGAG NCTGGGGCCA GATGGTAAGC CCTCCGTATC GTAGTTATCT CACAGCAGGG 240

AGTCAG 246

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

CTGGCACTGG AGCAGTGGGT CCCTGGTCTC CTACAAGTCC TGGGGCATTG GAGCCCCAAG 60

CAGTGTTAAT CCTGGCACTG TGTGAG 86

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 154 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

CTGGAGCCGG TCGAGCGTGG GTCTCGCGTA TCATTCGAGN CTGGGGCCAG ATGGTAAGCC 60

CTCCGTATCG TAGTTATCTC ACAGCCGTAT CATTCGAGNC TGGGGCCAGA TGGTAAGCCC 120

TCCGTATCGT AGTTATCTCA CAGCAGGGAG TCAG 154

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 221 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

AGGAGACTTG TGGTAAAAAT CTGCTGCTGT ACTGCTCATT TGGGAACCTT AGTATACTAA 60

ATAATATAAT ATATCAACAA CTAATGGTCA GCCAATGCTA TGCTGGATAT GAGGGTCCTG 120

GGCCACAAAG ACAAAAAATC AGGAACCACT TTTTAAGTGA GATACTTTGG GTCTCTGTCA 180

AATTCATAAC ACTTATTTCT TGGTGGAATA CAGTTAATGA G 221

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 231 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

AGGAGCGGGG AGGCACGATG GCCGCTTTGG TCCGGATCTT TGTGAGGAAC CTTACTTCTG 60

TGGTGTGACA TAATTGGACA AACTACCTAC AGAGATTTAA AGCTCTAAGG AAATATAAAA 120

TTTTTAAGTG TATAATGTGT TAAACTACTG ATTCTAATTG TTTGTGTATT TTAGATTCCA 180

ACCCTATGGA ACCTGATGAA TGGGAGCCAG TGGTGGAATG CCTTTAATGA G 231

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

TAAATCTGGA GCCGGTCGAG CGTGGGTCTC GCGTATCATT CGAGCACTGG GGCCAGATGG 60

TAAGCCCTCC GTATCGTAGT TATCTCACAG CAGGGAGTCA GG 102

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 110 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

TAGAACCGCC GCTGGCACTG GAGCAGTGGG TCCCTGGTCT CCTACAAGTC CTGGGGCATT 60

GGAGCCCCAA GCAGTGTTAA TCCTGGCTAC TGTGTGAGCC TGACCTCAAG 110

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

ATAGATATCT ACTTTATTCG ATTTAAATTC TGTTTAGTAT TTTATTATAT TTTGTTAATC 60

CATTTGTCCC AATTCATATA CTTAT 85

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

ATAGATAGCT ACTTAAAATA ACTTACACAC TGTTTTAGAG TGCTTGAAAA CTATCTGATC 60

AGACATAGTA ATTGAAACCA ATGAATACAT TATAT 95

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

GAAGGATCTG TTTGTAAGAA CGGAGCGGGA TACCATAAAA ATAGAGGTAA TAACATACAT 60

TGGGACGTGT AAATTTATTT TTATNNAANT 90

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

GAAGGATCTT ACCATTTTAA GAAAGGTCCA AAATTAAATA AAAATTTAAT TAGTCTCTTT 60

AGTGTTGCAT ACCATCAATA TCGGAATACT AAAT 94

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 200 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

ACCTGGTGCT GTGCTCACCC AGGCCGAGGG TGCCTTTGTG GCCTCACTGA TTAAGGAGAG 60

TGGCATGATG ACTTCAATGT CTGGATTGGC CTCCATGACC CCAAAAAGAA CCGCCGCTGG 120

GGAGCGTGGG TCCCTGGTCT CCTACAAGTC CTGGGGCATT GGAGCCCCAA GCAGTGTTAA 180

TCCTGGCTAC TGTGTGAGCC 200

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 218 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

AACTGGCGAA CTACTTACTC TAGCTTCCGG CAACAATTAA TAGACTGGAT GGAGGCGGAT 60

AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCCGGCTG GCTGGTTTAT TGCTGATAAA 120

TCTGGAGCCG GTGAGCGTGG GTCTCGCGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC 180

CCTCCGTATC GTGGTTATCT ACACGACGGG GAGTACGC 218

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 146 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

AGGCCCATCA TCAGTTTTTA TATAAAGAAA AAAAAACCTT AAAATTGTTA GGCAAATACT 60

ATGACAAATT GTAATATATA TTCTTACATT TCAGATTTTT ATTTTTTAAA CTGTATAGAA 120

TTGATTAATA AATAAAATTT AGTATT 146

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

AGGCACGATG GCCGCTTTGG TCCGGATCTT TGTGAAGGAA CCTTACTTCT GTGTGTGACA 60

TAATTGGACA AACTACCTAC AGAGATTTAA ACGTCTAAGG TAAATATAAA ATTTTTAGTG 120

TATAGGTTAA ACTACTGATT CTAATGTTGT GTATT 155

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGTATCATTG CAGCACTGGG GCCAGATGGT 60

AAGCCCTCCG TATCGTGGTT ATCTACACGA CGGGGAGTAC GG 102

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

TAGAACCGCC GCTGGCACTG GAGCAGTGGG TCCCTGGTCT CCTACAAGTC CTGGGGCATT 60

GGAGCCCCAA GCAGTGTTAA TCCTGGCTAC TGTGTGAGCC TGACCTCAAG CACAGG 116

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1418 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 14..1418

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

```
TTTTTTTTTT GAG ATG GAG TTT TCG CTC TTG TTG CCC AGG CTG GAG TGC    49
               Met Glu Phe Ser Leu Leu Leu Pro Arg Leu Glu Cys
                 1               5                  10

AAT GGC GCA ATC TCA GCT CAC CGC AAC CTC CGC CTC CCG GGT TCA AGC   97
Asn Gly Ala Ile Ser Ala His Arg Asn Leu Arg Leu Pro Gly Ser Ser
        15                  20                  25

GAT TCT CCT GCC TCA GCC TCC CCA GTA GGC TGG GAT TAC AGG CAT GTG  145
Asp Ser Pro Ala Ser Ala Ser Pro Val Gly Trp Asp Tyr Arg His Val
     30                  35                  40

CAC CAC GCT CGG CTA ATT TTG TAT TTT TTT TTA GTA GAG ATG GAG TTT  193
His His Ala Arg Leu Ile Leu Tyr Phe Phe Leu Val Glu Met Glu Phe
 45                  50                  55                  60

CTC CAT GTT GGT CAG GCT GGT CTC GAA CTC CGA CCT CAG ATG ATC CTC  241
Leu His Val Gly Gln Ala Gly Leu Glu Leu Arg Pro Gln Met Ile Leu
                65                  70                  75


CCG TCT CGG CCT CCC AAA GTG CTA GAT ACA GGA CTG AGC ACC ATG CCC  289
Pro Ser Arg Pro Pro Lys Val Leu Asp Thr Gly Leu Ser Thr Met Pro
            80                  85                  90

GGC CTC TGC CTG GCT AAT TTT TGT GGT AGA AAC AGG GTT TCA CTG ATG  337
Gly Leu Cys Leu Ala Asn Phe Cys Gly Arg Asn Arg Val Ser Leu Met
        95                  100                 105

TGC CCA AGC TGG TCT CCT GAG CTC AAG CAG TCC ACC TGC CTC AGC CTC  385
Cys Pro Ser Trp Ser Pro Glu Leu Lys Gln Ser Thr Cys Leu Ser Leu
    110                 115                 120

CCA AAG TGC TGG GAT TAC AGG CGT GCA GCC GTG CCT GGC CTT TTT ATT  433
Pro Lys Cys Trp Asp Tyr Arg Arg Ala Ala Val Pro Gly Leu Phe Ile
125                 130                 135                 140
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TTA TTT TTT TTA AGA CAC AGG TGT CCC ACT CTT ACC CAG GAT GAA GTG  481
Leu Phe Phe Leu Arg His Arg Cys Pro Thr Leu Thr Gln Asp Glu Val
                145                 150                 155

CAG TGG TGT GAT CAC AGC TCA CTG CAG CCT TCA ACT CTG AGA TCA AGC  529
Gln Trp Cys Asp His Ser Ser Leu Gln Pro Ser Thr Leu Arg Ser Ser
            160                 165                 170

ATC CTC CTG CCT CAG CCT CCC AAA GTA GCT GGG ACC AAA GAC ATG CAC  577
Ile Leu Leu Pro Gln Pro Pro Lys Val Ala Gly Thr Lys Asp Met His
        175                 180                 185

CAC TAC ACC TGG CTA ATT TTT ATT TTT ATT TTT AAT TTT TTG AGA CAG  625
His Tyr Thr Trp Leu Ile Phe Ile Phe Ile Phe Asn Phe Leu Arg Gln
    190                 195                 200

AGT CTC AAC TCT GTC ACC CAG GCT GGA GTG CAG TGG CGC AAT CTT GGC  673
Ser Leu Asn Ser Val Thr Gln Ala Gly Val Gln Trp Arg Asn Leu Gly
205                 210                 215                 220

TCA CTG CAA CCT CTG CCT CCC GGG TTC AAG TTA TTC TCC TGC CCC AGC  721
Ser Leu Gln Pro Leu Pro Pro Gly Phe Lys Leu Phe Ser Cys Pro Ser
                225                 230                 235

CTC CTG AGT AGC TGG GAC TAC AGG CGC CCA CCA CGC CTA GCT AAT TTT  769
Leu Leu Ser Ser Trp Asp Tyr Arg Arg Pro Pro Arg Leu Ala Asn Phe
            240                 245                 250

TTT GTA TTT TTA GTA GAG ATG GGG TTT CAC CAT GTT CGC CAG GTT GAT  817
Phe Val Phe Leu Val Glu Met Gly Phe His His Val Arg Gln Val Asp
        255                 260                 265

GCT AGA TCT CTT GAC CTT GTG ATC TGC CTG CCT CGG CCT CCC AAA GTG  865
Ala Arg Ser Leu Asp Leu Val Ile Cys Leu Pro Arg Pro Pro Lys Val
    270                 275                 280

CTG GGA TTA CAG GAC GTG ACG CCC ACC GCC CGG CCT ATT TTT AAT TTT  913
Leu Gly Leu Gln Asp Val Thr Pro Thr Ala Arg Pro Ile Phe Asn Phe
285                 290                 295                 300

TGT TTG TTT GAA ATG GAA TCT CAC TCT GTT ACC CAG GCT GGA GTG CAA  961
Cys Leu Phe Glu Met Glu Ser His Ser Val Thr Gln Ala Gly Val Gln
                305                 310                 315
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TGG CCA AAT CTC GGC TCA CTG CAA CCT CTG CCT CCC GGG CTC AAG CGA 1009
Trp Pro Asn Leu Gly Ser Leu Gln Pro Leu Pro Pro Gly Leu Lys Arg
            320                 325                 330

TTC TCC TGT CTC AGC CTC CCA AGC AGC TGG GAT TAC GGG CAC CTG CAC 1057
Phe Ser Cys Leu Ser Leu Pro Ser Ser Trp Asp Tyr Gly His Leu His
        335                 340                 345

CAC ACC CCG CTA ATT TTT GTA TTT TCA TTA GAG GCG GGG TTT CAC CAT 1105
His Thr Pro Leu Ile Phe Val Phe Ser Leu Glu Ala Gly Phe His His
    350                 355                 360

ATT TGT CAG GCT GGT CTC AAA CTC CTG ACC TCA GGT GAC CCA CCT GCC 1153
Ile Cys Gln Ala Gly Leu Lys Leu Leu Thr Ser Gly Asp Pro Pro Ala
365                 370                 375                 380

TCA GCC TTC CAA AGT GCT GGG ATT ACA GGC GTG ACG CCT CAC CCA GCC 1201
Ser Ala Phe Gln Ser Ala Gly Ile Thr Gly Val Thr Pro His Pro Ala
                385                 390                 395

GGC TAA TTT AGA TAA AAA AAT ATG TAG CAA TGG GGG GTC TTG CTA TGT 1249
Gly

TGC CCA GGC TGG TCT CAA ACT TCT GGC TTC ATG CAA TCC TTC CAA ATG 1297

AGC CAC AAC ACC CAG CCA GTC ACA TTT TTA AAC AGT TAC ATC TTT ATT 1345

TTA GTA TAC TAG AAA GTG ATA CGA TAA CAT GGC GGA ACC TGC AAA TTC 1393

GAG TAG TAC AGA GTC TTT TAT AAC T                                1418
```

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 402 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

Met Glu Phe Ser Leu Leu Leu Pro Arg Leu Glu Cys Asn Gly Ala Ile
1 5 10 15

Ser Ala His Arg Asn Leu Arg Leu Pro Gly Ser Ser Asp Ser Pro Ala
20 25 30

Ser Ala Ser Pro Val Gly Trp Asp Tyr Arg His Val His His Ala Arg
35 40 45

Leu Ile Leu Tyr Phe Phe Leu Val Glu Met Glu Phe Leu His Val Gly
50 55 60

Gln Ala Gly Leu Glu Leu Arg Pro Gln Met Ile Leu Pro Ser Arg Pro
65 70 75 80

Pro Lys Val Leu Asp Thr Gly Leu Ser Thr Met Pro Gly Leu Cys Leu
85 90 95

Ala Asn Phe Cys Gly Arg Asn Arg Val Ser Leu Met Cys Pro Ser Trp
100 105 110

Ser Pro Glu Leu Lys Gln Ser Thr Cys Leu Ser Leu Pro Lys Cys Trp
115 120 125

Asp Tyr Arg Arg Ala Ala Val Pro Gly Leu Phe Ile Leu Phe Phe Leu
130 135 140

Arg His Arg Cys Pro Thr Leu Thr Gln Asp Glu Val Gln Trp Cys Asp
145 150 155 160

His Ser Ser Leu Gln Pro Ser Thr Leu Arg Ser Ser Ile Leu Leu Pro
165 170 175

Gln Pro Pro Lys Val Ala Gly Thr Lys Asp Met His His Tyr Thr Trp
180 185 190

Leu Ile Phe Ile Phe Ile Phe Asn Phe Leu Arg Gln Ser Leu Asn Ser
195 200 205

Val Thr Gln Ala Gly Val Gln Trp Arg Asn Leu Gly Ser Leu Gln Pro
210 215 220

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Leu Pro Pro Gly Phe Lys Leu Phe Ser Cys Pro Ser Leu Leu Ser Ser
225                 230                 235                 240

Trp Asp Tyr Arg Arg Pro Pro Arg Leu Ala Asn Phe Phe Val Phe Leu
                245                 250                 255

Val Glu Met Gly Phe His His Val Arg Gln Val Asp Ala Arg Ser Leu
            260                 265                 270

Asp Leu Val Ile Cys Leu Pro Arg Pro Pro Lys Val Leu Gly Leu Gln
        275                 280                 285

Asp Val Thr Pro Thr Ala Arg Pro Ile Phe Asn Phe Cys Leu Phe Glu
    290                 295                 300

Met Glu Ser His Ser Val Thr Gln Ala Gly Val Gln Trp Pro Asn Leu
305                 310                 315                 320

Gly Ser Leu Gln Pro Leu Pro Pro Gly Leu Lys Arg Phe Ser Cys Leu
                325                 330                 335

Ser Leu Pro Ser Ser Trp Asp Tyr Gly His Leu His His Thr Pro Leu
            340                 345                 350

Ile Phe Val Phe Ser Leu Glu Ala Gly Phe His His Ile Cys Gln Ala
        355                 360                 365

Gly Leu Lys Leu Leu Thr Ser Gly Asp Pro Pro Ala Ser Ala Phe Gln
    370                 375                 380

Ser Ala Gly Ile Thr Gly Val Thr Pro His Pro Ala Gly
390                 395                 400
```

What Is Claimed Is:

1. A method for detecting the presence of Neural Thread Protein (NTP) having a molecular weight of about 8 kDa, about 14 kDa, about 17 kDa, about 21 kDa, about 26 kDa or about 42 kDa, in a human subject, said method comprising:

(a) contacting a biological sample from said human subject that is suspected of containing said NTP with at least one molecule capable of binding to said protein; and

(b) detecting any of said molecule bound to said protein.

2. The method of claim 1, wherein said molecule is selected from the group consisting of:

(a) an antibody substantially free of natural impurities;

(b) a monoclonal antibody; and

(c) a binding fragment of (a) or (b).

3. The method of claim 1, wherein the detecting of any of said molecule bound to said protein is performed by *in situ* imaging.

4. The method of claim 1, wherein the detecting of any of said molecule bound to said protein is performed by *in vitro* imaging.

5. The method of claim 1, wherein said molecule is administered to said human subject.

6. The method of claim 1, wherein said molecule is bound to said protein *in vivo*.

7. A method of diagnosing the presence of Alzheimer's Disease in a human subject suspected of having Alzheimer's Disease which comprises:

(a) incubating a biological sample from said subject which is suspected of containing NTP having a molecular weight of about 8 kDa, about 14 kDa, about 17 kDa, about 21 kDa, about 26 kDa or about 42 kDa, in the presence of at least one binding molecule capable of identifying said NTP; and

(b) detecting said binding molecule which is bound in said sample, wherein said detection indicates that said subject has Alzheimer's Disease.

8. The diagnostic method of claim 7, wherein said detection is by immunometric assay.

9. The diagnostic method of claim 8, wherein said immunometric assay is a monoclonal antibody-based immunometric assay.

10. The diagnostic method of claim 7, wherein said method comprises:

(a) incubating said biological sample with two different NTP monoclonal antibodies bound to a solid phase support; and

(b) detecting NTP bound to said monoclonal antibodies with a third different detectably labeled NTP monoclonal antibody in solution.

11. The diagnostic method of claim 7, wherein said incubating step further includes adding a known quantity of labeled Neural Thread Protein whereby a competitive immunoassay is established.

12. The diagnostic method of claim 7, wherein said detection is by immuno-polymerase chain reaction.

13. A method of diagnosing the presence of neuroectodermal tumors in a human subject suspected of having a neuroectodermal tumor which comprises:

(a) incubating a biological sample from said subject which is suspected of containing NTP having a molecular weight of about 8 kDa, about 14 kDa, about 17 kDa, about 21 kDa, about 26 kDa or about 42 kDa, in the presence of at least one binding molecule capable of identifying said NTP; and

(b) detecting said binding molecule which is bound in said sample, wherein said detection indicates that said subject has a neuroectodermal tumor.

14. The diagnostic method of claim 13, wherein said detection is by an immunometric assay.

15. The diagnostic method of claim 14, wherein said immunometric assay is a monoclonal antibody-based immunometric assay.

16. The diagnostic method of claim 13, wherein said method comprises:

(a) incubating said biological sample with two different NTP monoclonal antibodies bound to a solid phase support; and

(b) detecting NTP bound to said monoclonal antibodies with a third different detectably labeled NTP monoclonal antibody in solution.

17. The diagnostic method of claim 13, wherein said incubating step further includes adding a known quantity of the corresponding labeled NTP whereby a competitive immunoassay is established.

18. The diagnostic method of claim 13, wherein said detection is by immuno-polymerase chain reaction.

19. A method of diagnosing the presence of a malignant astrocytoma in a human subject suspected of having a malignant astrocytoma which comprises:

(a) incubating a biological sample from said subject which is suspected of containing NTP having a molecular weight of about 8 kDa, about 14 kDa, about 17 kDa, about 21 kDa, about 26 kDa or about 42 kDa, in the presence of at least one binding molecule capable of identifying said NTP; and

(b) detecting said binding molecule which is bound in said sample, wherein said detection indicates that said subject has a malignant astrocytoma.

20. A method of diagnosing the presence of a glioblastoma in a human subject suspected of having glioblastomas which comprises:

(a) incubating a biological sample from said subject suspected of containing NTP having a molecular weight of about 8 kDa, about 14 kDa, about 17 kDa, about 21 kDa, about 26 kDa or about 42 kDa, in the presence of at least one binding molecule capable of identifying said NTP; and

(b) detecting said binding molecule which is bound in said sample, wherein said detection indicates that said subject suffers from a glioblastoma.

21. A Neural Thread Protein (NTP) substantially free of any natural impurities and having a molecular weight of about 42 kDa, 26 kDa, 21 kDa, 17 kDa, 14 kDa, or 8 kDa.

22. The NTP according to claim 21, wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:121.

23. An isolated nucleic acid m lecule coding for the NTP according to Claim 21.

24. The nucleic acid molecule according to claim 23, wherein the molecule comprises the nucleic acid sequence set forth in SEQ ID NO:120 which encodes the amino acid sequence set forth in SEQ ID NO:121.

25. The nucleic acid molecule according to claim 23, wherein the molecule encodes the amino acid sequence set forth in SEQ ID NO:121.

26. The nucleic acid molecule of claim 23 which is a plasmid.

27. An expression vector comprising the nucleic acid molecule of claim 23.

28. A host cell transformed with the plasmid of claim 26.

29. A method of using the plasmid of claim 26 to prepare an NTP, said method comprising:

(a) introducing said plasmid into a host cell to produce a recombinant host cell;

(b) culturing said recombinant host cell; and

(c) isolating said NTP from said recombinant host cell.

30. A nucleic acid probe for the detection of the presence of NTP in a DNA sample from an individual comprising a nucleic acid molecule sufficient to specifically detect under stringent hybridization conditions the presence of the molecule according to claim 23 in said sample, wherein said probe is nonhomologous to a PTP nucleic acid sequence.

31. The probe according to claim 30, wherein said probe is a 15- to 30-mer antisense ligonucleotide which is complementary to an NTP nucleic acid sequence and which is nonhom logous to a PTP nucleic acid sequence.

32. A method of detecting the presence of a genetic sequence coding for NTP having a molecular weight of about 8 kDa, about 14 kDa, about 17 kDa, about 21 kDa, about 26 kDa or about 42 kDa, in a sample, which comprises:

(a) contacting said sample with the probe of claim 30 under conditions of hybridization; and

(b) detecting the formation of a hybrid of said probe and said sequence.

33. A method of producing an NTP having a molecular weight of about 8 kDa, about 14 kDa, about 17 kDa, about 21 kDa, about 26 kDa or about 42 kDa, said method comprising:

(a) culturing a recombinant host comprising a human gene coding for said NTP; and

(b) isolating said NTP from said host.

34. The method of claim 33, wherein said host is *E. coli*.

35. The method of claim 33, wherein said gene is contained by a vector.

36. A substantially pure NTP having a molecular weight of about 42 kDa, about 26 kDa, about 21 kDa, about 17 kDa, about 14 kDa, or about 8 kDa obtained by the process of claim 33.

37. A pharmaceutical composition comprising the probe according to claim 31 and a pharmaceutically acceptable carrier.

38. A ribozyme comprising a target sequence which is complementary to an NTP sequence and nonhomologous to a PTP nucleic acid sequence.

39. A nucleic acid molecule which codes for the ribozyme of claim 38.

40. A pharmaceutical composition comprising the NTP ribozyme of claim 38 and a pharmaceutically acceptable carrier.

41. A method for inhibiting the expression of an NTP in a patient, said method comprising administering to said patient an effective amount of the antisense oligonucleotide of claim 31.

42. A method for inhibiting the expression of NTP in a patient, said method comprising administering to said patient an effective amount of the ribozyme of claim 38.

43. A method for inhibiting the expression of an NTP in a patient, said method comprising administering to said patient an effective amount of the DNA molecule of claim 39.

44. An oligonucleotide comprising the sequence 3'X5'-L-5'X3', wherein X comprises an NTP nucleic acid sequence which is nonhomologous to the PTP nucleic acid sequence, and wherein L represents an oligonucleotide linkage.

45. An oligonucleotide comprising the sequence 5'X3'-L-3'X5', wherein X comprises an NTP nucleic acid sequence which is nonhomologous to the PTP nucleic acid sequence, and wherein L represents an oligonucleotide linkage.

46. A method to treat diseases or conditions mediated by the presence f an NTP having a molecular weight of about 8 kDa, 14 kDa, 17 kDa, about 21 kDa, about 26 kDa or about 42 kDa, which method comprises

administering to a patient in need of such treatment an effective amount of the oligonucleotide of claims 44 or 45, or a pharmaceutical composition thereof.

47. A ribonucleotide NTP external guide nucleic acid comprising:

(a) a 10-15 nucleotide sequence which is complementary to an NTP nucleic acid sequence and which is nonhomologous to the PTP nucleic acid sequence; and

(b) a 3'-NCCA nucleotide sequence, wherein N is a purine.

48. A method to treat diseases or conditions mediated by the presence of an NTP having a molecular weight of about 8 kDa, about 14 kDa, about 17 kDa, about 21 kDa, about 26 kDa or about 42 kDa, which method comprises administering to a patient in need of such treatment an effective amount of the ribonucleotide NTP external guide nucleic acid according to claim 47, or a pharmaceutical composition thereof.

49. A virion comprising the expression vector of claim 27.

50. A method to treat diseases or conditions mediated by the abnormally low level of expression of an NTP having a molecular weight of about 8 kDa, 14 kDa, 17 kDa, about 21 kDa, about 26 kDa or about 42 kDa, which method comprises administering to a patient in need of such treatment an effective amount of the virion of claim 49.

51. A method of differentiating between sporadic and familial Alzheimer's Disease in a human subject, said method comprising:

(a) obtaining a biological sample from said human subject who is suspected of having Alzheimer's Disease;

(b) purifying DNA from said biological sample; and

(c) contacting said DNA with the probe of claim 30 under conditions of hybridization;

wherein familial Alzheimer's Disease is indicated by the detection of a hybrid of said probe and said DNA, and

wherein sporadic Alzheimer's Disease is indicated by the absence of detection of hybridization.

52. An antibody having binding affinity to the NTP of claim 21 but not to a PTP.

53. A hybridoma which produces the monoclonal antibody according to claim 52.

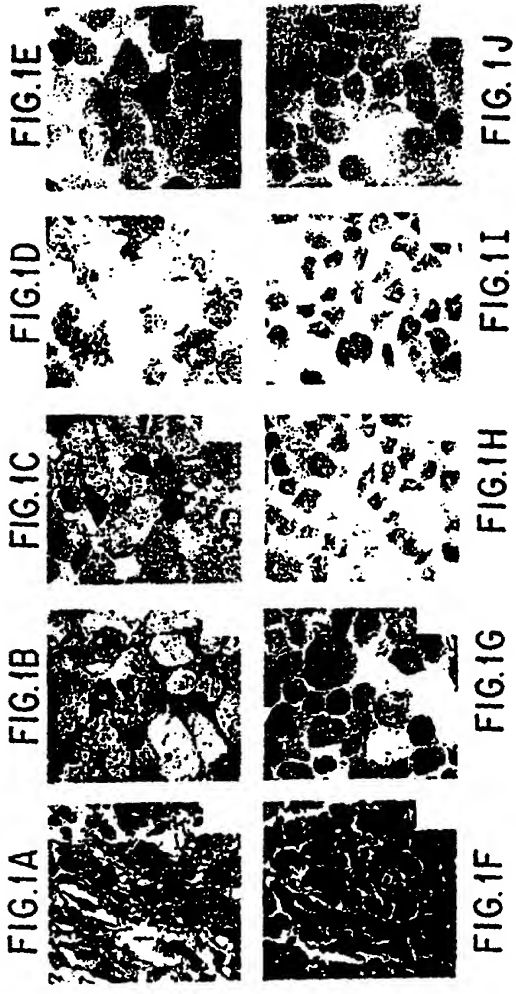


FIG.1A
FIG.1B
FIG.1C
FIG.1D
FIG.1E
FIG.1F
FIG.1G
FIG.1H
FIG.1I
FIG.1J

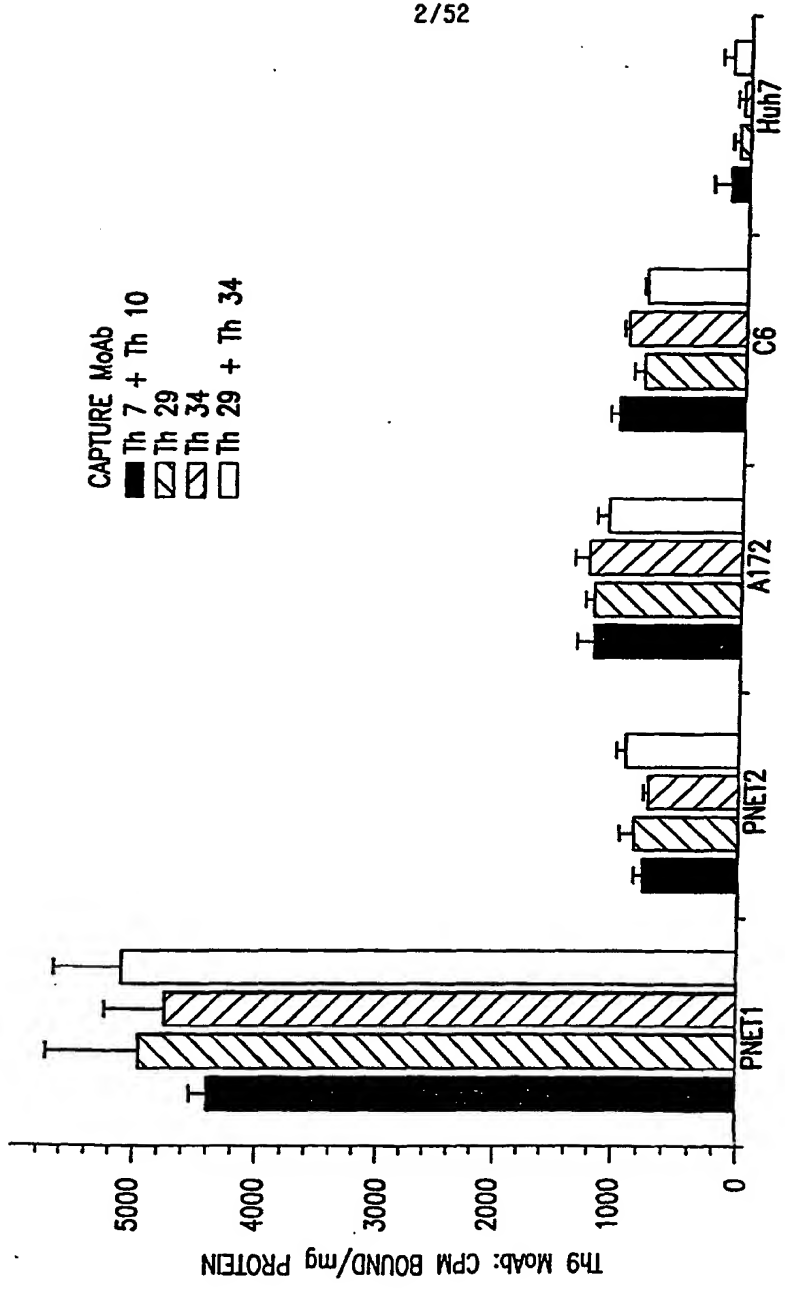

CAPTURE MoAb
Th 7 + Th 10
Th 29
Th 34
Th 29 + Th 34
Th9 MoAb: CPM BOUND/mg PROTEIN
0
1000
2000
3000
4000
5000
PNET1
PNET2
A172
C6
Huh7

FIG.2

FIG. 3

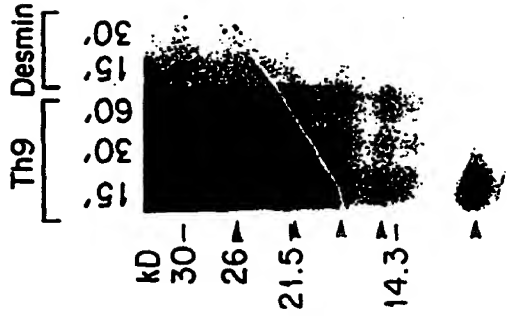

Th9
Desmin
15'
30'
60'
90'
120'
NO
kD
30
26
21.5
14.3

FIG.4B

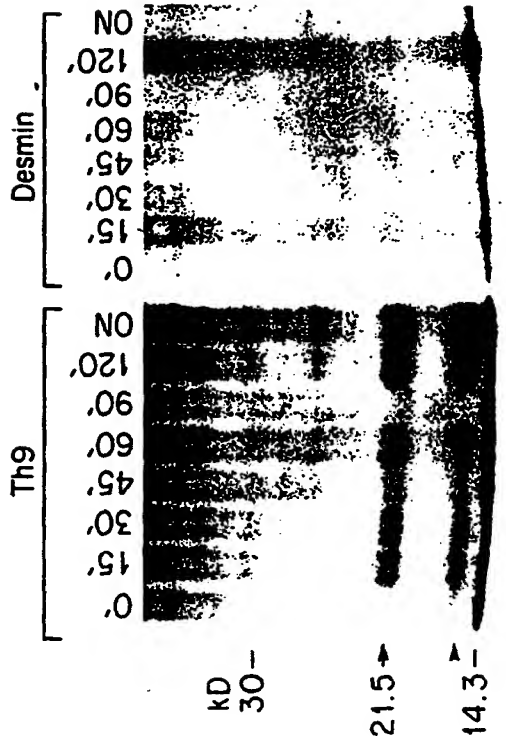

Th9
Desmin
0'
15'
30'
45'
60'
90'
120'
NO
kD
30
21.5
14.3

FIG.4A

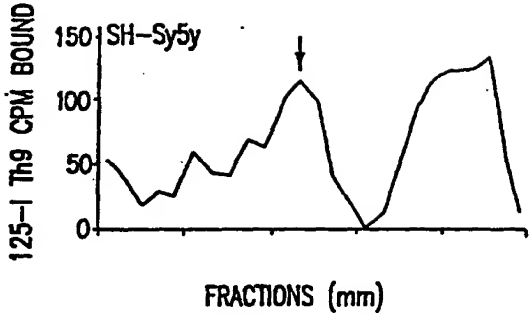


FIG.5A

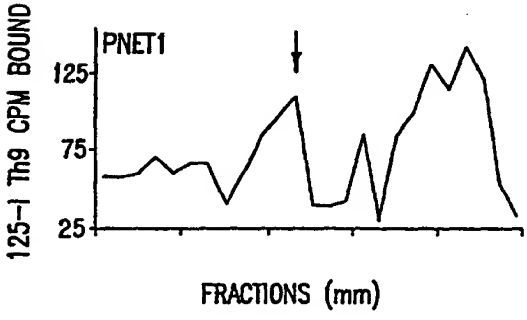


FIG.5B

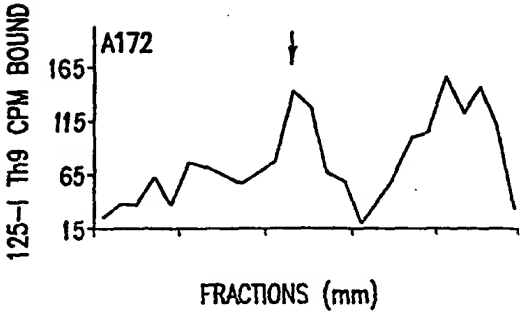


FIG.5C

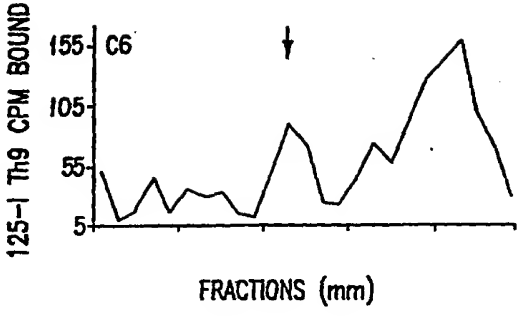

125-I Th9 CPM BOUND
155
105
55
5
C6
FRACTIONS (mm)

FIG.5D

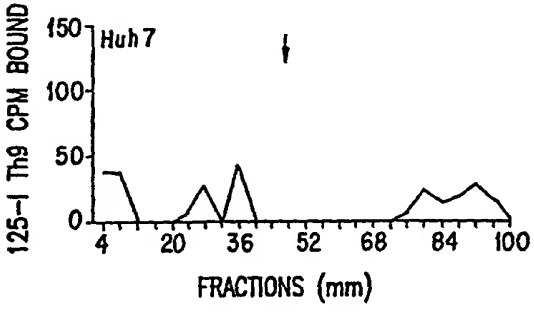

125-I Th9 CPM BOUND
150
100
50
0
Huh7
4
20
36
52
68
84
100
FRACTIONS (mm)

FIG.5E

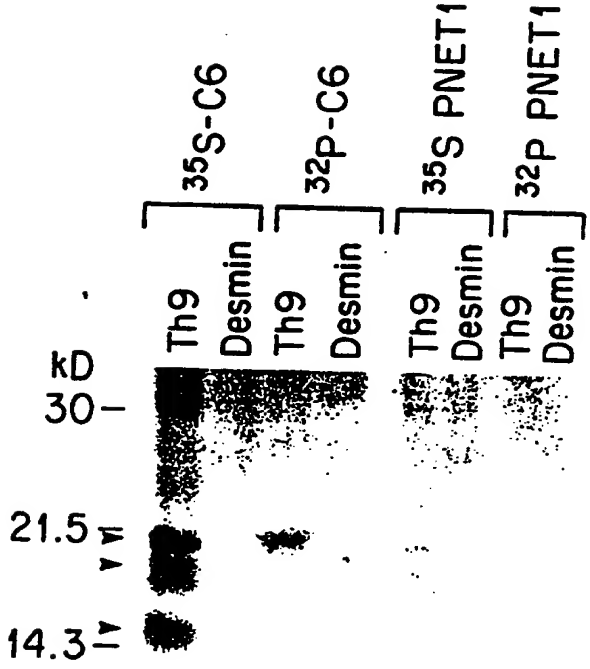

35S-C6
32P-C6
35S PNET1
32P PNET1
Th9
Desmin
Th9
Desmin
Th9
Desmin
Th9
Desmin
kD
30
21.5
14.3

FIG. 6

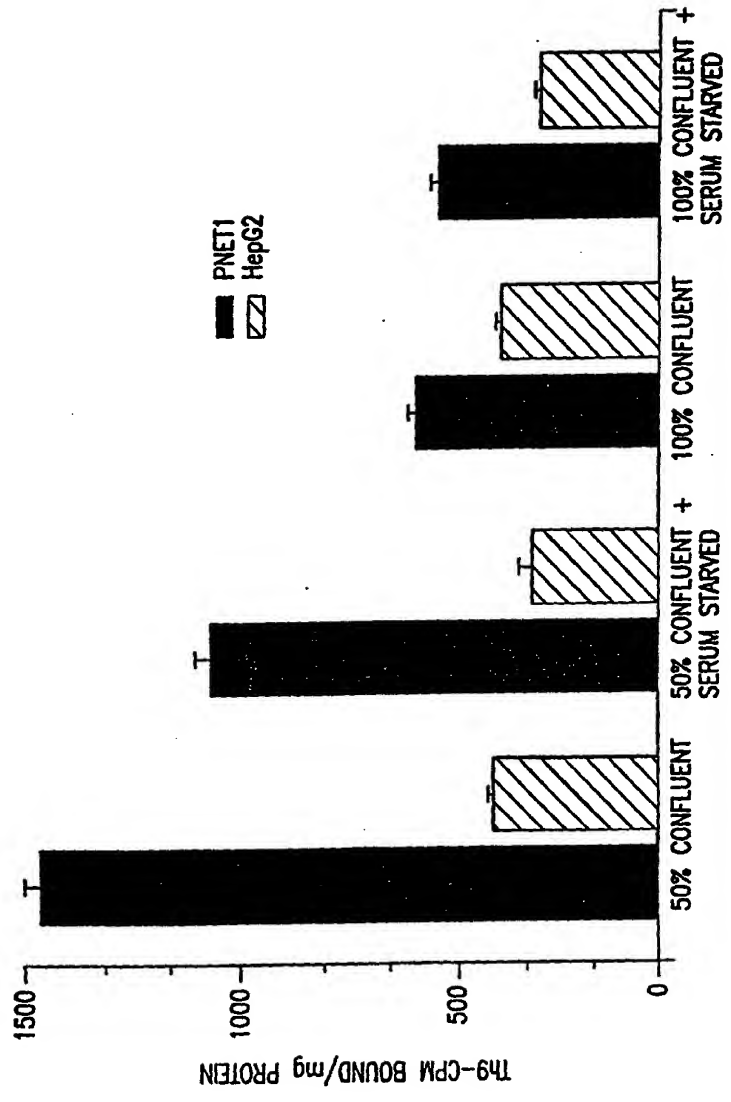

PNET1
HepG2
1500
1000
500
0
I125-CPM BOUND/mg PROTEIN
50% CONFLUENT
50% CONFLUENT + SERUM STARVED
100% CONFLUENT
100% CONFLUENT + SERUM STARVED

FIG.7

FIG. 8A

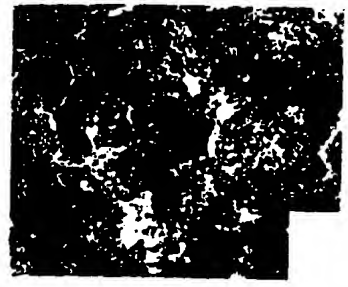


FIG. 8B

FIG. 8C

FIG. 8D

FIG. 8E

FIG. 8F

1-9a T7 SEQUENCE

Sequence Range: 1 to 1442

```
CGCTG CGCCC AGGCT GGCTC TGGAA AGCCT GTGCG GTCCT GGCAG GAAGC CCGGC CCGTG   60
GAGCA GGTTT TCGTT CTGCT TCAGC AATAA ATAAG GGTGA CCACA GGGAC TTTGC TTTTG  120
GTTTC CTTTC CTGTG AAAAG GTTGG TTTTA AAGTG AGATA CACTT TTCCG TAGAA CAAGT  180
GTTCT ATCTT TAAAA ACCCA AATTG CAGCA CCGTG GATTA CTGGT CTCAG AACAA CTCAT  240
TGCGC ATCAG ATTTG ACTCT CTGAT TTTCT GTCTA TTGGC CAAAT TGCCC TTTAA CTGCA  300
CCTGA ATCCT TTGTG TACTG ATGCC TTTGA GCTGG GCACC TTGGG AGAGT GTTGT GTTGC  360
TGTTT ACGGT TCTTC CTTCC CCTTG CTAAT TACAG TCTCT GGTGC CCAGC AAGCC CCTTT  420
GGCTT CCTTC CGTGA CTGGT CACGT TGTCT GCCTG GGCTC AGCGT GGACC TGCCC CATGC  480
TGCAG AACCT GGCCT CACCT GGACT TTTAC TAGAA TTGCC AGCTT CTCAA CTTAG CAGAT  540
CATCA CTCAT GCGGG CACAA GCAAA GATCA ACACT TTCTT TTTTG GTAAG CTTGA GTTTT  600
ACAAG TTATT TTTTG GTGAT GCGTA AGACA TTGCA GTGGG AAACC ATTCA ACTTG AGTTT  660
ATTGG AGTTT GCTGT TGTAG CAGGT TTTAA CTCAG GAACA ACTCT TGTCT GATCT CTCGC  720
CCCTC TGCCG GGACT ACATT ACTGT CTCTC GGAGC CGGTA GCGTT GCTGT CGAGT CCCAG  780
GACTA TCTCT GCAGA CTGCT ATGCT CAGAT CGAAG TATTT CACAA GAATA CTTGT GTTTT  840
TAACA GCCCT TCCCC TGGAC GGTGC GCCAT GAGGG CCTCA TGTTA CGCAT TGCCT TTTCT  900
TTCTG TGGAT CCAGT ATCTT CCTCG GCTTT TTAGG GAGCA GGAAA AATGC GTCTG AGAGC  960
AACTC TTTTT AAAAA CCTGC CCTGT TGTAT ATAAC TGTGT CTGTT TCACC GTGTG ACCTC 1020
CAAGG GGGTG GGAAC TTGAT ATAAA CGTTT AAAGG GCCCA CGATT TGCCC GAGGG TTACT 1080
CCTTT GCTCT CACCT TGTAT GGATG AGGAG ATGAA GCCAT TTCTT ATCCT GTAGA TGTGA 1140
AGCAC TTTCA GTTTT CAGCG ATGTT GGAAT GTAGC ATCAG AAGCT CGTTC CTTCA CACTC 1200
AGTGG CGTCT GTGCT TGTCC ACATG CGCTG GGCGT CTGGA CCTTG AATGC CTGCC CTGGT 1260
TGTGT GGACT CCTTA ATGCC AATCA TTTCT TCACT TCTCT GGACA CCCAG GGCGC CTGTT 1320
GACAA GTGTG GAGAA ACTCC TAATT TAAAT GTCAC AGACA ATGTC CTAGT GTTGA CTACT 1380
ACAAT GTTGA TGCTA CACTG TTGTA ATTAT TAAAC TGATT ATTTT TCTTA TGTCA AAAAA 1440
AAA
```

FIG.9

WP5' SEQUENCE

Sequence Range: 1 to 313

```
GATCC CGTTT GACAG GTGTA CCGCC CCAGT CAAAC TCCCC ACCTG GCACT GTCCC CGGAG
CGGTC GCGCC CGCGC GACCA CGGAG CTCTG GGCGC CAGAA GCGAG AGCCC CTCGC TGCCC
CCCGC CTCAC CGGGT AGTGA AAAAA CCATG AGAGT AGTGG TATTT CACCG GCGGC CCGCG
AGGAC CCCCG CCCGA CCCAG TGCGG AACGG GGG
```

FIG.9A

```
9A•1-T7                                                    5   10   15   20
[ 386 ]   ————————————————————————————————————————————CAcc GtgGa TtA-C tgGtC>
Human-PTP                                                  CATT GCAGC TCAGC ATGGC

9A•1-T7    25   30   35   40   45   50   55   60   65   70   75
[ 386 ]   TCAGA aCAaC TCATt gcgCA T-CaG ATtTa CTctC TGAtt TTTCT GTCTa ttgGC CAttG>
Human-PTP TCAGA CCAGC TCATA CTTCA TGCTG ATCTC CTGCC TGATG TTTCT GTCTC AGAGC CAAGG

9A•1-T7  85   90   95   100  105  110  115       125  130      135  140
[ 386 ]   CCctt taaCt gcacC tGA-a TcCtt tgtGt aCtGA TCctt TGagC tG—G GCACC -tTG->
Human-PTP CCAAG AGCCC CAGAC AGAGT TGCCC CAGGC CCGGA TCAGC TGCCC AGAAG GCACC AATGC

9A•1-T7    145      150  155  160  165  170  175  180  185  190  195
[ 386 ]   ggAga G-T-g TtgTG tTgCT -gTTT AcgGt tcttC cT-tc cCCTt GcTaa tTaCA G-TCT>
Human-PTP CTATC GCTCC TACTG CTACT ACTTT AATGA AGACC GTGAG ACCTG GGTTG ATGCA GATCT

9A•1-T7    200  205  210  215  220  225  230  235  240  245  250  255
[ 386 ]   CTggT GCCAG cA-Ag ccccT ttGGC ttCCT tccGT gacTG gTCAC gttGt CtGcc tGgGC>
Human-PTP CTATT GCCAG AACAT GAATT CGGGC AACCT GGTGT CTGTG CT Q C CCAGG CCGAG GGTGC

9A•1-T7    260  270  275  280  285  290      300  305  310  315
[ 386 ]   CagcG TGCCC cCA-T GcTgc A-GAa ccTGG C-CTc AgGAC TTttc acT-a GaATT GcCCT>
Human-PTP CTTTG TGCCC TCACT GATTA AGGAG AGTGG CACTG ATGAC TTCAA TGTCT GGATT GGCCT

9A•1-T7   325  330  335  340  345  350  355  360  365  370  375
[ 386 ]   tCcTc A-aCt tAgcA GAtCa ttCaC Tcatg CgGGc aCA-a Gcaaa gaTca aCaCt TtC-t>
Human-PTP CCATG ACCCC AAAAA GAACC GCCGC TGGCA CTGGA GCAGT GGGTC CCTGG TCTCC TACAA

9a-t7                      10   15   20   25   30       35       40   45
 [ 180 ]   ——————————TT tTCCT AgA-A cA-aG -gGtT cTatC T-TTA A—AA -Accc aaATT>
H REG GENE               TT CTCCT ATAGA GATTG TTGAT TTGCC TCTTA AGCAA GAGAT TCATT

9a-t7       50   55 65   70   75   80   85   90   95 100  105  110
 [ 180 ]   GCAGC aCcGC tgGtC TCAGA aCAaC TCATt gcgCA T-CaG ATtTa CTctC TGATt TTTCT>
H REG GENE GCAGC TCAGC ATGCC TCAGA CCAGC TCATA CTTCA TGCTG ATCTC CTGCC TGATG TTTCT

9a-t7     115  120  125  130  135  140  145  150  155
[ 180 ]   GTCTa tttGg CcAaa T-tGc cCT-T TTaaC tgCAc CtgAa TCTTT>
H REG GENE GTCTC AGAGC CAAGG TAAGA TCTCT TTTCC ACCAA CCAAC TCTTT
```

FIG.10

```
9A•1-T7                                        5   10   15   20   25   30
[ 130 ]  ————————————————————————————————CAc cGtgG aTtA- CtgGt CTCAG AoCAo>
EXON2                                          CAT TGCAG CTCAG CATGG CTCAG ACCAG

9A•1-T7     35   40   45   50   55   60   65   70   75   80
[ 130 ]  CTCAT tgcgC AT-Co GATtT aCTct CTGAT tTTTC TGTCT attgG CCAA>
EXON2    CTCAT ACTTC ATGCT GATCT CCTGC CTGAT GTTTC TGTCT CAGAG CCAA

H REG GENE                5   10   15   20   25   30   35   40   45
[ 136 ]  ————————G aAtTc cTGgg cTCAo GtgAt CCTC- TCatG caGTC TCC— CA-aA gT-GC>
WP03-4 T7          G GAGTT TTGTC ATCAG GCCAG CCTCA TCCCG AGGTC TCCTC CACCA TTGGC

H REG GENE  50   55   60   65   70   75   80   85   95   100
[ 136 ]  tG-gG atgaC AGGcT -tGaG C-CAC C-AcA —ccA ggCCC aT-Co TCAGt ttatA TAAAG>
WP03-4 T7  CGTAG CCAGC AGGTT CAGTG CTCAC CGAAA GTAAA ATCCC CTCCT TCAGC AAGAA TAAAG

H REG GENE  105  110  115  120  125  130
[ 136 ]  aAAaA aAaAC CTTAa aaT-t gtTAg GcAA- ATA>
WP03-4 T7  CAATA TACAC CTTAG GTTCC ACTAA GTAAC ATA

WP03 BSP          120  115  110  105  100   95   90       85   80   75
[ 108 ]  ———<GTT-c TgTGa gTCTc AAttt gttcc TTCtT gGaAG CT—G tcTGG -TgA- AtCtG
EXON2          GTTGA TTTGC CTCTT AAGCA AGAGA TTCAT TGCAG CTCAG CATGG CTCAG ACCAG

WP03 BSP    70   65   60   55   50   45   40   35        30
[ 108 ]  <tTggT cCcTC tgtCT GcToT tCTGt CTG-T cTgTa TGTCT —G-t CCAtG
EXON2     CTCAT ACTTC ATGCT GATCT CCTGC CTGAT GTTTC TGTCT CAGAG CCAAG
```

FIG.10A

```
AD3-4-296                    260  255  250      245      240  235  230
[ 112 ]  ————————————————<ACtA CcaAa C-CT- -GCal tA-AA —aAa ttlCg gTlG- -GlCg
WP5'4/93                      ACCA CGGAG CTCTG GGCGC CAGAA GCGAG AGCCC CTCGC TGCCC

AD3-4-296 225    220    215  210  205  200  195    190  185  180  175  170
[ 112 ]    <aCCtC -ggAg C-aGa AcccA Acctc CGAgc AGtac A-TGc TAaga CttCa cCaGt CaaaG
WP5' 4/93   CCCGC CTCAC CGGGT AGTGA AAAAA CGATG AGAGT AGTGG TATTT CACCG GCGGC CCGCG

AD3-4-296      165  160  155  150    145  140  135  130  125  120  115
[ 112 ]   <-cGA- aCgta CtatA CtCAa T—tG AtCca ataAc TtGaC CaacG Gaaca AgTTA ccCTA
WP5' 4/93   AGGAC CCCCC CCCGA CCCAG TGCGG AACGG GGGAG TAGTC CCGGG GGCTC ACTTA TTCTA

AD3-4-296   105   100   95   90   85   80   75 70   65   60   55   50
[ 112 ]   <tAacA G-CgC Aatcc tAttC TAGAG TCcAt aTCAA CAGGG TtTaC gacCt CGaTG -TTgg
WP5' 4/93   CATTA GTCTC ACGTG CAGAC TAGAG TCAAG CTCAA CAGGG TCTTC TTTCC CGCTG ATTCC


AD3-4-296    45   40
[ 112 ]   <atCAg GaC
WP5' 4/93   GCCAA GTC

AD2 SP6F        10   15 20   25   30   35   40   45   50   55   60   65
[ 504 ]    AG-Tt TCaCT CTGTt gCCCA GGCTG gAGTG CAaTG GCaCA ATCct GGCTC ACTGC aAcCT>
1-9AT7-3 3 AGATC TCGCT CTGTC ACCCA GGCTG AAGTG CAGTG GCCCA ATCTC GGCTC ACTGC GAGCT

AD2 SP6F      70   75   80   85   90   95  100      105      110  115
[ 504 ]    CCgCC TCCCG aGcTC Aagca ATTCT CCTGC CTCA- -GCCT C-G— —TGA GccGC TGGGA>
1-9AT7-3 3 CCACC TCCCG GGTTC ACTTC ATTCT CCTGC CTCAC TGCCT CAGCC TCTGA GTAGC TGGGA
```

FIG.10B

HB4-SEQ SEQUENCE

GAGGC GTATT ATACC ATGCT CCATC TGCCT ACGAC AAACA GACCT AAAAT CGCTC ATTGC 60
ATACT CTTCA ATCAG CCACA TAGCC CTCGT AGTAA CAGCC ATTCT CATCC AAACC CCCTG 120
AAGCT TCACC GGCGC AGTCA TTCTC ATAAT CGCCC ACGGG CTTAC ATCCT CATTA CTATT 180
CTGCC TAGCA AACTC AAACT ACGAA CGCAC TCACA GTCGC ATCAT AATCC TCTCT CAAGG 240
ACTTC AAACT CTACT CCCAC TAATA GCTTT TTGAT GACTT CTAGC AAGCC TCGCT AACCT 300
CGCCT TACCC CCCAC TATTA ACCTA CTGGG AGAAC TCTCT GTGCT AGTAA CCACG TTCTC 360
CTGAT CAAAT ATCAC TCTCC TACTT ACAGG ACTCA ACATA CTAGT CACAG CCCTA TACTC 420
CCTCT ACATA TTTAC CACAA CACAA TGGGG CTCAC TCACC CACCA CATTA ACAAC ATAAA 480
ACCCT CATTC ACACG AGAAA ACACC CTCAT GTTCA TACAC CTATC CCCCA TTCTC CTCCT 540
ATCCC TCAAC CCCGA CATCA TTACC GGGTT TTCCT CTTAA AAAAA AAAAA AAAA 590

HB4 PROTEIN

EAYYT MLHLP TTNRP KIAHC ILFNQ PHSPR SNSHS HPNPL KLHRR SHSHN RPRAY ILITI 60
LPSKL KLRTH SQSHH NPLSR TSNST PTNSF LMTSS KPR 95

FIG.11A

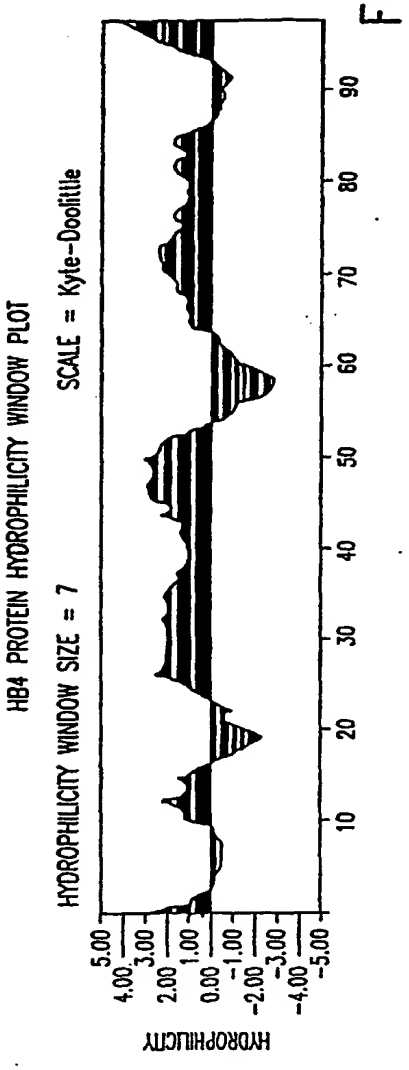


FIG.11B

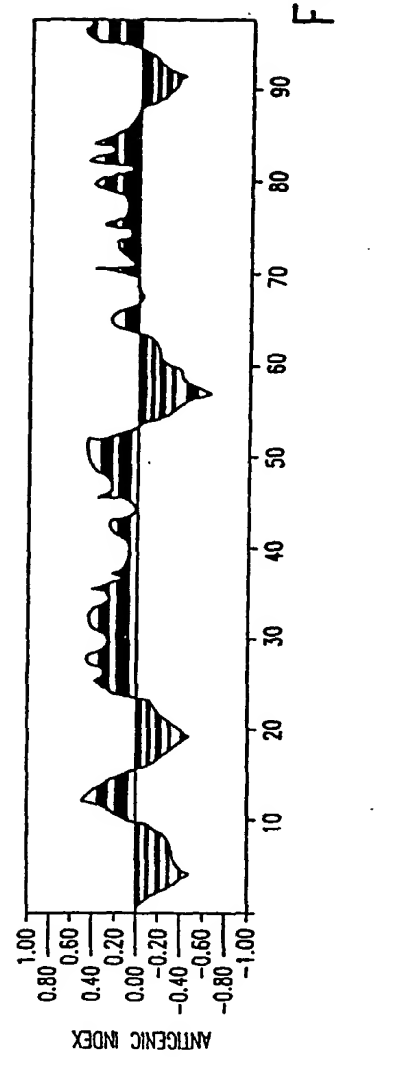


FIG.11C

```
HB4 SEQ
[ 440 ]  ————————————————————————————————————————ATaC>
Human-PTP                                          ATGC

HB4-SEQ 15    20   25    30   35    40   45    50   55   60   65
[ 440 ]  C-AT- GCTCC atCTG C—CT ACgac AA-ac AGACC -T-A- AaaTc GcTca tTGCA tA-CT>
Human-PTP CTATC GCTCC TACTG CTACT ACTTT AATGA AGACC GTGAG ACCTG GGTTG ATGCA GATCT

HB4-SEQ    70   75   80   85    90   95   100  105  110  115  120
[ 440 ]  CTtca atCAG cACAT -Agcc CtcG- tAgta acaG- CcaTt CTCAt CCAaa CCccc tGaag>
Human-PTP CTATT GCCAG AACAT GAATT CGGGC AACCT GGTGT CTGTG CTCAC CCAGG CCGAG GGTGC

HB4-SEQ 125  130  135  140  145  150  155  160  165  170  175
[ 440 ]  CTTca ccGgC gCAgT cATT- ctcAt AaTcG C-Cca cgGgC TTacA T-cCT -cATT actaT>
Human-PTP CTTTG TGGCC TCACT GATTA AGGAG AGTGG CACTG ATGAC TTCAA TGTCT GGATT GGCCT

HB4-SEQ 180  185 190  195  200  205  210   215  220  225 230  235
[ 440 ]  tC-TG cCaqC AAAct cAAaC taCGa acGCA CT-cA -CAGT cGcat CaTaa TCTCt ctCAA>
Human-PTP CCATG ACCCC AAAAA GAACC GCCGC TGGCA CTGGA GCAGT GGGTC CCTGG TCTCC TACAA

HB4-SEQ 240   245  250  255  265 270 275  280  285 290  295  300  305
[ 440 ]  GgaCT -tcaa AcTct ActCC CAAGC ttTGT gAcTt CTaGC aACct cGctA aCCTc gCCTt>
Human-PTP GTCCT GGGGC ATTGG AGCCC CAAGC AGTGT TAATC CTGGC TACTG TGTGA GCCTG ACCTC

HB4-SEQ 310  315  320  325  330  340   345  350   355  360365  370
[ 440 ]  AccCc CActA TTaac ctAcT GGgAG aATGT G-CTa GT-AA -cCAc GTTCT CCTTc aaaTa>
Human-PTP AAGCA CAGGA TTCCA GAAAT GGAAG GATGT GCCTT GTGAA GACAA GTTCT ccTTT GTCTG

HB4-SEQ  375  380  385  390   395  400  405  410  415  420  425
[ 440 ]  tcAcT ctcct ActTA cAGG- A-CT- cAAcA TACta GTCcA GccCT -ATaC tcCct cTACA>
Human-PTP CAAGT TCAAA AACTA GAGGC AGCTG GAAAA TACAT GTCTA GAACT GATCC AGCAA TTACA

HB4-SEQ 430  435  440  445  450  455  460  465  470 475  480  485
[ 440 ]  tattt acCAc AAcac AAtgg GGctC A-CTC aCCcA C-CAc aTtAA CCata AaACc CTCaT>
Human-PTP ACGGA GTCAA AAATT AAACC GGACC ATCTC TCCAA CTCAA CTCAA CCTGG ACACT CTCTT

HB4-SEQ 490  495  500  505  510  515  520   525  530   540  545
[ 440 ]  -TCac acGAG -aaaa Caccc TcATg TTC-A TAcac cTA— TcCCC CAtTC TTcct AtCCc>
Human-PTP CTCTG CTGAG TTTGC CTTGT TAATC TTCAA TAGTT TTACC TACCC CAGTC TTTGG AACCT

HB4-SEQ  550  555  560 565  570  575  580  585  590
[ 440 ]  TcAAc cccgA cAtcA AcCgg GTTTC CtCTt AAAAA AAAAA AAAAA A>
Human-PTP TAAAT AATAA AAATA AACAT GTTTC CACTA AAAAA AAAAA AAAAA A
```

FIG.11D

```
H REG GENE      2260  2265  2270  2275  2280  2285  2290  2295  2300  2305
[ 284 ]    ——C tTcTT -TttC AgGC- CaAgo gGCCc A-GAC AgAgt tgCC- ccAgg CcCgg ATcag>
HB4-SEQ      C GTATT ATACC ATGCT CCATC TGCCT ACGAC AAACA GACCT AAAAT CGCTC ATTGC

H REG GEN2310  2315  2320  2322330  2335  2340  2345  2350  2355  2360  2365  2370
[ 284 ]    cTgCc CagaA ggCAc CaACc TAtCg CTCcT AcT-g CtaCt AcTtT aATgo AgACC gCgaG>
HB4-SEQ    ATACT CTTCA ATCAG CCACA TAGCC CTCGT AGTAA CAGCC ATTCT CATCC AAACC CCCTG

H REG GBNE     2375  2380  23852390  2395  2400 2405  2410  2415  2420  2425  2430
[ 284 ]    -AcCT gggtt GatGC AGTgt gagTg AggAg aGCgt gtGGG aaggg AgaCT CATgA -aggg>
HB4-SEQ    AAGCT T QCC GGCGC AGTCA TTCTC ATAAT CGCCC ACGGG CTTAC ATCCT CATTA CTATT

H REG GENE      2435  2440  2445  2450  2455  2460  2465 2470  2475  2480  2485
 [ 284 ]   agGgg aAGC- tgC-C ActCT -CcAg tGtgt TCAgt GgCGC Aatgo gAT-g agaCT gAAcc>
HB4-SEQ    CTGCC TAGCA AACTC AAACT ACGAA CGCAC TCACA GTCGC ATCAT AATCC TCTCT CAAGG

H REG GENE 24902495  2500  2505  2510  2515  2520  2525  2530  2535  2540
[ 284 ]    cCTTt AtACT aTcaT CagcC ccA-A aCTTT ccaAT —CTa CT—t tAtCC -CatT AttCa>
HB4-SEQ    ACTTC AAACT CTACT CCCAC TAATA GCTTT TTGAT GACTT CTAGC AAGCC TCGCT AACCT

H REG GENE2545  2550  2555  2560  2565  2570  2580  2585  2590  2595  2600
[ 284 ]    gcaCa TtCCC agCAC aAagA ACCTg gTGGG tG-AC agcaT catC- AcggA Catta cTCTg>
HB4-SEQ    CGCCT TACCC CCCAC TATTA ACCTA CTGGG AGAAC TCTCT GTGCT AGTAA CCACG TTCTC

H REG GEN2605  2610  2612620 2625  2630  2635  2640  2645  2650  2655  2665  2670
[ 284 ]    CTG-T CttlT tTCAC cCTCC T-CTT ggAGG ACTCA gtATA tccGT CACAa CCCTc cACTg>
HB4-SEQ    CTGAT CAAAT ATCAC TCTCC TACTT ACAGG ACTCA ACATA CTAGT CACAG CCCTA TACTC

H REG GENE  2675  2680    2685    2690    2695  2700    2705  2710  2715    2720
[ 284 ]    agTCT cCAT- TTT-C ttC— tgCAA ca—G CTCta T-tgC CAgaA CATgA A-ttC gggcA>
HB4-SEQ    CCTCT ACATA TTTAC CACAA CACAA TGGGG CTCAC TCACC CACCA CATTA ACAAC ATAAA

H REG GENE 2725   2730   2735    2740
[ 284 ]    A-CCT -ggTg tC-tG tG—c tCACC C>
HB4-SEQ    ACCCT CATTC ACACG AGAAA ACACC C
```

FIG.11E

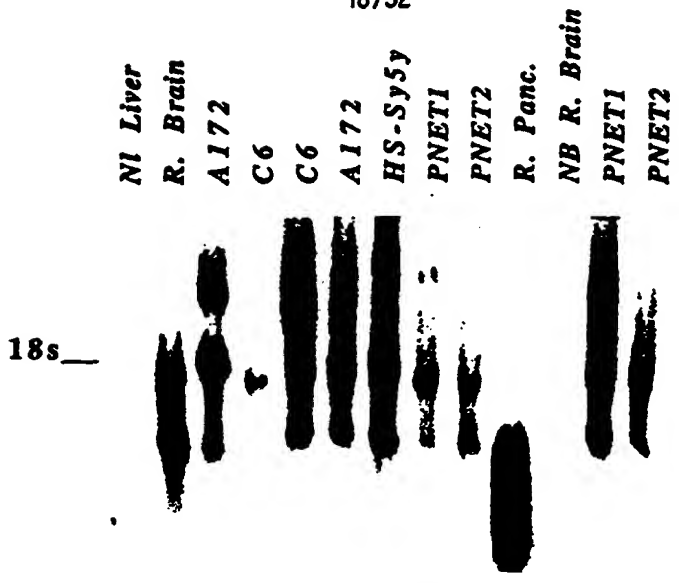


FIG. 12A

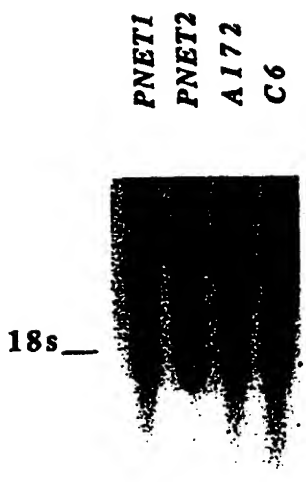


FIG. 12B

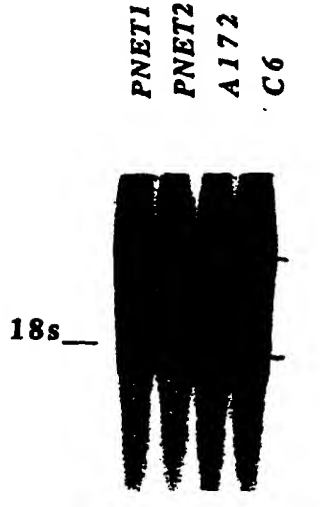


FIG. 12C

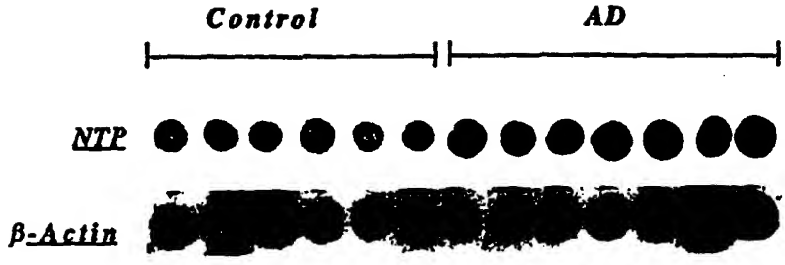


FIG. 13A

FIG. 13B

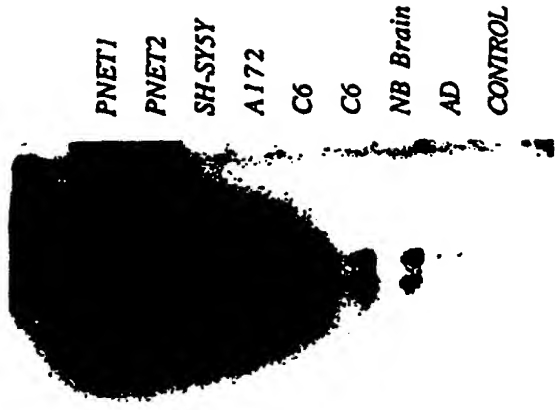
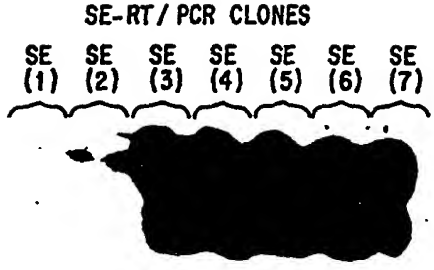


FIG. 14A

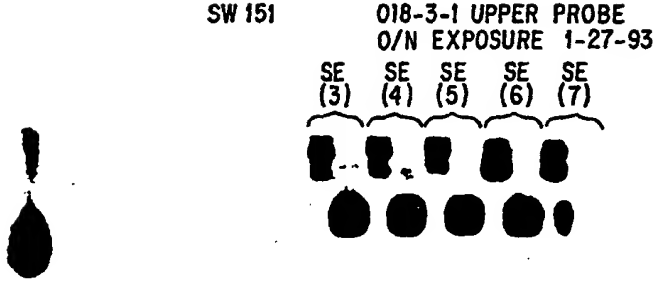
FIG. 14B 1-9a

FIG. 14C



1-9a

FIG. 15A



0-18

FIG. 15B

AD2-2 T7

```
GTTCT TAGTC TATCT CTTGT ACAAA CGATG TGCTT TGAAG ATGTT AGTGT ATAAC AATTG   60
ATGTT TGTTT TCTGT TTGAT TTTAA ACAGA GAAAA AATAA AAGGG GGTAA TAGCT CCTTT  120
TTTCT TCTTT CTTTT TTTTT TTCAT TTCAA AATTG CTGCC AGTGT TTTCA ATGTA GGACA  180
ACAGA GGGAT ATGCT GTAGA GTGTT TTTAT TGCCT AGTTG ACAAA GCTGC TTTTG AATGC  240
TGGTG GTTCT ATTCC TTTGC ACATC ACGAC ATTTT ATAAT CATAG TTAAA TCGTA TATGA  300
CAAAA ATGCT CTGAT CTGAT GCCAA AGGTC AATTC AGTGT ATATA ACCTG AACAC ACTCA  360
TCCAT TGCGT TT                                                           372
```

AD2-2 T7 PEP

```
MFVFC LILNR EKIKG GNSSF FLLSF FFSFQ NCCQC FQCRT TEGYA VECFY CLVDK AAFEC  60
WWFYS FDT
```

FIG.16A

AD2 SP6F

```
ACTGT CTCCC CCTTT GATAG GGACA CTAAA GTGGT CTGTA CTTGG GTAGA GGATG GCANG   60
TTAAG AATTA AAATC GTCTG GGTGC GGTCT GCACG CTTGT AATCC CAGCA CTTTG GGAGG  120
CTGAG GCGGG CGGAT CACCT GAGGT CAGGA GTTCG ACACC AGCCT GATGA ACATG GAGAA  180
ACCCC ATCTC TACTA AAAAT ACAAA TATTA GCTGG GCGTT GTCGC GCGCC TGTAA TCCCA  240
GCGGC TCACG AGGCT GAGGC AGGAG AATTG CTTGA GCTCG GGATG GCGGA GGTTG CAGTG  300
AGCCA GGATT GTGCC ATTGC ACTCC AGCCT GGGCA ACAAG AGTGA AACTC TGTCT CAAAA  360
AAAAA AAAAA AAAAA AA                                                     377
```

FIG.16D

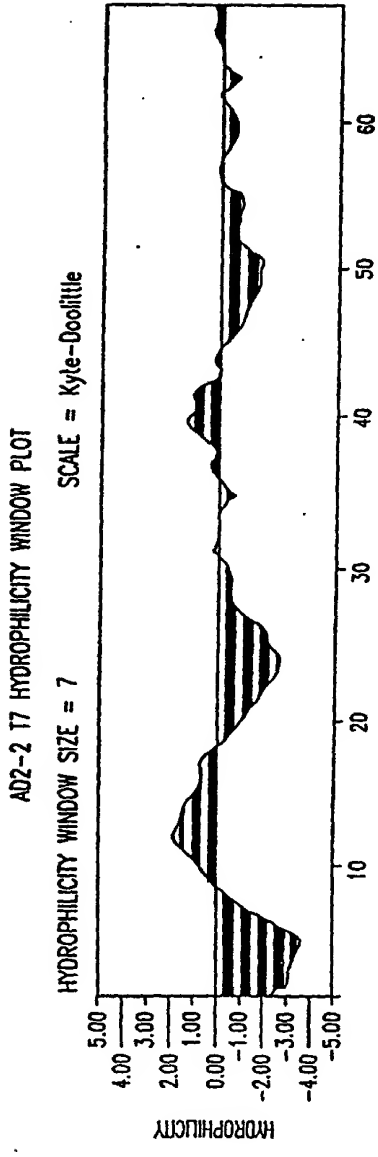


FIG.16B

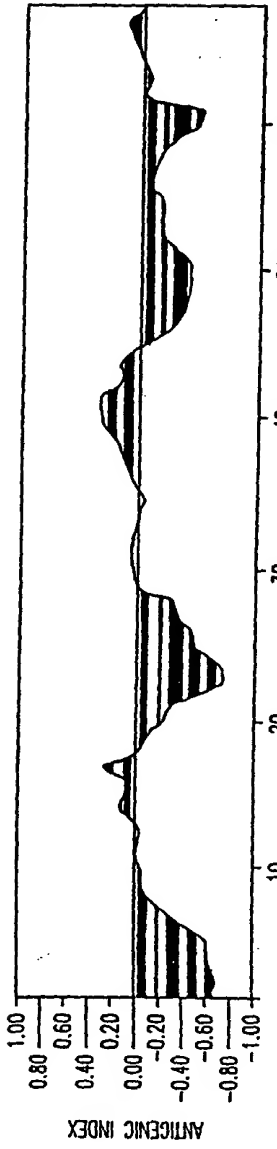


FIG.16C

AD2-2 SEQUENCE

GCGTAAACAC ATTTTTGTTC TTAGTCTATC TCTTGTACAA ACGATGTGCT TTGAAGATGT 60
TAGTGTATAA CAATTGATGT TTGTTTTCTG TTTGATTTTA AACAGAGAAA AAATAAAAGG 120
GGGTAATAGC TCCTTTTTTC TTCTTTCTTT GATTTTAAAC AGAGAAAAAA TAAAACGGGG 180
TAATAGCTCC TTTTTTCTTC TTTCTTTTTT TTTTTTCATT TCAAAATTGC TGCCAGTGTT 240
TTCAATGATG GACAACAGAG GGATATGCTG TAGAGTGTTT TATTGCCTAG TTGACAAAGC 300
TGCTTTGAAT GCTGGTGGTT CTATTCCTTT GACACTACGC ACTTTTATAA TACATGTTAA 360
TGCTATAGGA CAAGATGCTC TGATTCCTGA GTGCCAGAGG TTCAATTCAG TGTATATAAC 420
TGAACACACT CATCCATTTG TGCTTTTGTT TTTTTTATGC TGGCTTAAAG GTAAAGAGCC 480
CATCCTTTGC AAGTCATCCA TGTTGTTACT TAGGCATTTT ATCTTGGCTC AAATTGTTGG 540
AAGAATGGTG GCTTGTTTCA TGGTTTTTGT ATTTGTGTCT AATGCACGTT TTAACATGAT 600
AGACGCAATG CATTGTGTAG CTAGTTTTCT GGAAAAGTCA ACTCTTTTAG GAATTGTTTT 660
TCAGATCTTC AATAAATTTT TTCTTTAAAT TTCAAAGAAC AATGTGCTTG TGTTGATGCC 720
TTACAAAAAC CATTGTATAT TTGTGTATTC CTTCTTGTAT TTAGACAGTG GTTTTTCAGG 780
TGCGTGCTTT GTTTTCTGGT ATGGCCTTTA TGGAATGAGA CGCTTTAGCT TTGGTACGTA 840
GCGCTAATCC ATAGCAGCTT TGGCAGTTTC GTGTCTTGAG TCTTAGCTAA AAAGTTAGAA 900
GTTTACATGA CTGTTTTTTT TATTTTCCCT AAATTATTAC TTACTCTGAG CATTAATTAA 960
GGGCATTTTC ACCTGTGTAA AATTATGGTC AGCTTTTTTC TGTCTATAAT TGTTTACTTT 1020
TGTGGGTTTA CTCTAGAAAC ATGAGCCAAA AATGTCAATA GACAACACAG TATTAAAATA 1080
ACCCAAAAGT TGTAAAGGGC AACGTTTCTC CCCTTTGATA GGGACACTAA AGTGGTCTGT 1140
ACTTGGGTAG AGGATGGCAG ACGTTAAGAA TTAAAATGCG TCTGGGTGCG GTCTCACGCT 1200
TGTAATCCCA GCACTTTGGG AGGCTGAGGC GGGCGGATCA CCTGAGGTCA GGAGTTCGAC 1260
ACCAGCCTGA TGAACATGGA GAAACCCCAT CTCTACTAAA AATACAAATA TTAGCTGGGC 1320
GTTGTCGCGC GCCTGTAATC CCAGCGGCTC ACGAGGCTGA GGCAGGAGAA TTGCTTGAGC 1380
TCGGGATGGC GGAGGTTGCA GTGAGCCAGG ATTGTGCCAT TGCACTCCAG CCTGGGCAAC 1440
AAGAGTGAAA CTCTGTCTCA AAAAAAAAAA AAAAAAAAAA 1480

FIG,16E

AD3-4 SEQUENCE

ttgggtgoggtggo

ATGAT GGTCT GTTGG AATCG GTTTG GTAAA TGGGT TTATT TCATA TCCGC TATCT TTAAC 60
TTTGG ACCGC GTTAT CTATA TCATG GCGTT CCTTT CTACT TTTTA ATATT GGTTC GTATT 120
ATATC GTTCC TGATT GGGGA TATGG AAGAC GTATT ACTTA ATTGT ACTTT ATTGA AACGT 180
TCCTC TCGGT TTCGA TTCTG GGGGC TTTGG TCTGC TCGAT GGATT CTTGT CGATT TTCTC 240
GTGTG GCAGT AACAT ACCGT TTTAT CACCC TTCTA AATAT CCCAT CTCCC GCTGT TTGGT 300
AGGCT CGGAA CACTA TCGAC CAACA GGTTC TATCT AGAAT CAAGT TGGAA ATTAA ACGGT 360
GTCTT GG 367

AD3-4 PROTEIN

MMVCW NRFGK WVYFI SAIFN FGPRY LYHGV PFYFL ILVRI ISFLI GDMED VLLNC TLLKR 60
SSRFR FWGAL VCSMD SCRFS RVAVT YRFIT LLNIP SPAVW MARNT IDQQV LSRIK LEIKR 120
CL 122

FIG.16F

AD3-4T7

CCCAC AGGTC CTAAA CTACC AAACC TGCAT TAAAA AATTT CGGTT GGTCG ACCTC GGAGC 1180
AGAAC CCAAC CTCCG AGCAG TACAT GCTAA GACTT CACCA GTCAA AGCGA ACGTA CTATA 1240
CTCAA TTGAT CCAAT AACTT GACCA ACGGA ACAAG TTACC CTAGG GATAA CAGCG CAATC 1300
CTATT CTAGA GTCCA TATCA ACAAT AGGGT TTACG ACCTC GATGT TGGAT CAGGA CATCC 1360
CGATG GTGCA GCCGC TATTA AAGGT TCGTT TGTTC AAACG ATTAA AGTCC TCGTG TCTGA 1420
GTTCA GACCG AAGTA ATCCA GGTCG GTTTC TATCT TCTTC AAATT CCTCC CTGTA CCGAA 1480
AGGAC TAATG AGAAA TAAGG CCTAC TTCAC AAAGC GGCCT TCCCC CGTAA TGATA TCATC 1540
TCAAC TTAGT ATTAT ACCCA CACCC ACCCA AGAAC AGGGT TTGTT AAAAA AAAAA AAAAA 1600

FIG.16I

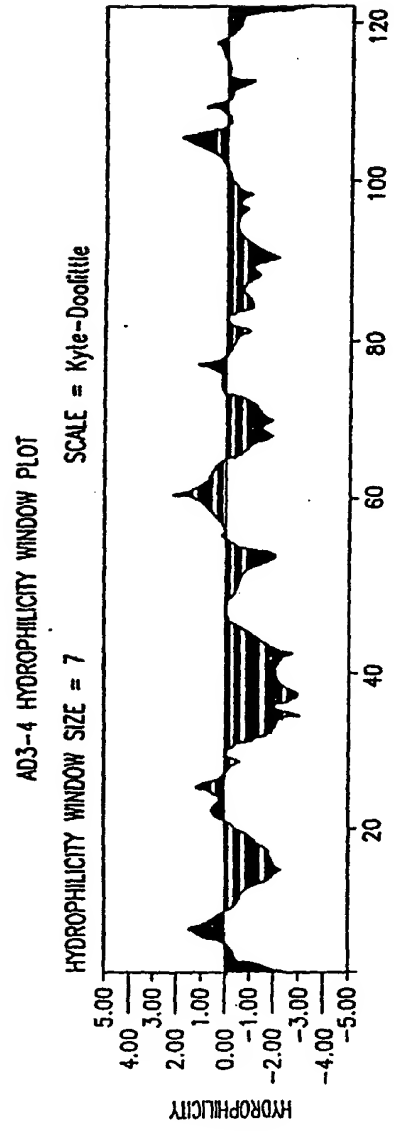


FIG.16G

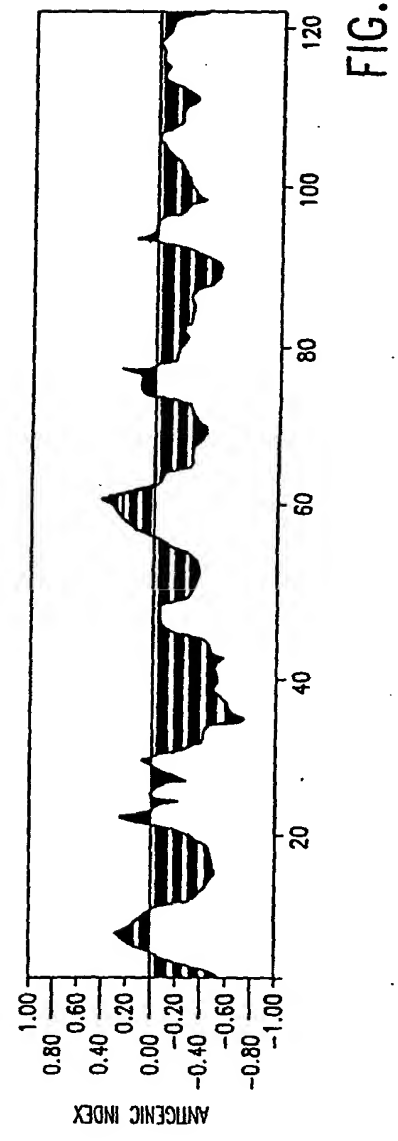


FIG.16H

AD3-4SP SEQUENCE

AACCCACTCC ACCTTACTAC CAGACAACCT TAGCCAAACC ATTTACCCAA ATAAAGTATA 60
GGCGATAGAA ATTGAAACCT GGCGCAATAG ATATAGTACC GCAAGGAAAG ATGAAAAATT 120
ATAACCAAGC ATAATATAGC AAGGACTAAC CCCTATACCT TCTGCATAAT GAATTAACAT 180
GAAATAACTT TGCAAGGAGA GCCAAAGCTA AGACCCCCGA AACCAGACGA GCTACCTAAG 240
AACAGCTAAA AGAGCACACC GTCATTGTAT GGCAAAATAG TGGGAAGATT TATAGGGTAG 300
AGGGCGACAA ACCATCCGAG CCTTGTGATA GCTGGTTGTC CAAGATAGAT CTTAGTTCAA 360
CCTTTAATTT GCCACAGAAC C 381

FIG.16J

AD3-4T7 SEQUENCE

TTTTTTTTTT TTTTTAACAA ACCCTGTTCT TGGGTGGGTG TGGGTATAAT ACTAAGTTGA 60
GATGATATCA TTACGGGGGA AGGCCGCTTT GTGAAGTAGG CCTTATTTCT CATTAGTCCT 120
TTCGGTACAG GGAGGAATTT GAAGAAGATA GAAACCGACC TGGATTACTT CGGTCTGAAC 180
TCAGACACGA GGACTTTAAT CGTTTGAACA AACGAACCTT TAATAGCGGC TGCACCATCG 240
GGATGTCCTG ATCCAACATC GAGGTCGTAA ACCCTATTGT TGATATGGAC TCTAGAATAG 300
GATTGCGCTG TTATCCCTAG GGTAACTTGT TCCGTTGGTC AAGTTATTGG ATCAATTGAG 360
TTTAGTAGTC CGCTTGGAGT GGTGAAGTCT AGAATGTCCT GTTCGGGGGT TGGTTTCTGC 420
TCCCAGGTCG CCCCAACCGA ATTTTTTATT GAAGGTTGGG TAGTTTAGCA CCTGTGGGTT 480
GGTAAGGTAC TGTTGGAATT AATAAATTAA AGCTCCATAG GGTCTCCTCG TCTTGTTGTG 540
TAATGCCCCC CTCTCCACGG GAAGGTCAAT TCCACTGGTT AAAAGTAAGA GAAAGCTGAA 600
CCCTCGGGGA GCCATCCATA CAGGTCCCC 629

FIG.16K

AD4-4 SP6 SEQUENCE

Sequence Ronge: 1 to 256

GCGGC TAAAT TGGTT TGTTA TTTTT TAAAA AAAAC TTGCA TGTTT AAAAA AAAGT TGATT 60
GCTTC AAATT TCTGC TACTA ACTTC AAGCT ATGGG AGTTT GGCAG TAGTC ACTTG AGGAT 120
TTTTT TTCCA ATTCT TTTCT TTTTG TTGTT AAAGC TGTAC TTCAG TGAAC AGAAA AATTG 180
CCAAG CAAAC TAATG GACTA TAAAG CGTAA TTTGA CTGTG TGGGA CTAAA CTACA GAGCC 240
TACTT GACCA GTGGA T 256

FIG.16L

AD4-4 T7F SEQUENCE

Sequence Range: 1 to 270

```
CATGT TTAAA AAAAA GTTGA TTGCT TCAAA TTACT GCTAC TAACT TCAAG CTATG GGAGT  60
TTGGC AGTAG TCACT TGAGG ATTTT TTTTC CAATT CGTTT TCATT TTTGT TGTTA AAGCT 120
CGTAC TTCAG TGAGA CAGAA AAATT GCCAA GCTAA ACTAA TGGTC TATAA AAGCG TAATT 180
TGCAT GTGTG GGCAA AAACT ACAGA GCCTC AATTG CCACT GAGGT ATAGT ACAAA GTTTT 240
AATAC ATTTT GTAAA TCAAA TTGAA AGAAA                                     270
```

FIG.16M

AD4-4 SEQUENCE

```
CATGTTTAAA AAAAAGTTGA TTGCTTCAAA TTACTGCTAC TAACTTCAAG CTATGGGAGT  60
TTGGCAGTAG TCACTTGAGG ATTTTTTTTC CAATTCGTTT TCATTTTTGT TGTTAAAGCT 120
CGTACTTCAG TGAGACAGAA AAATTGCCAA GCTAAACTAA TGGTCTATAA AAGCGTAATT 180
TGCATGTGTG GGCAAAAACT ACAGAGCCTC AATTGCCACT GAGGTATAGT ACAAAGTTTT 240
AATACATTTT GTAAATCAAA TTGAAAGAAA                                   270
```

FIG.16N

AD16c-T7 SEQUENCE

```
TCTGC CCAGG CTGGT CTGAA ATTCC TGGGC TGAAG TGATC CTCCA GTCTT GGCCT CCCAA  60
AGTGC TGGGA TTACA GGCAT GAGCT ACTGA GCCTA GCCTT AATGA TTAAT TTTAG AGTGA 120
TGGCT TGTAC CTTCA AGACA CATAT AGATT GAGAC AGAAA ATTTC CATCG TCCCC GAGAA 180
AACT                                                                   184
```

AD16c-T7 PEP

```
                5   10   15   20   25   30   35   40   45   50   55   60
SSSLG LPKCW DYRHE LLSLA LMINF RVMAC TFKQH IELRQ KISIV PRKLC CMGPV CPVKI

               65   70   75
ALLTI NGHCT WLPAS
```

FIG.160

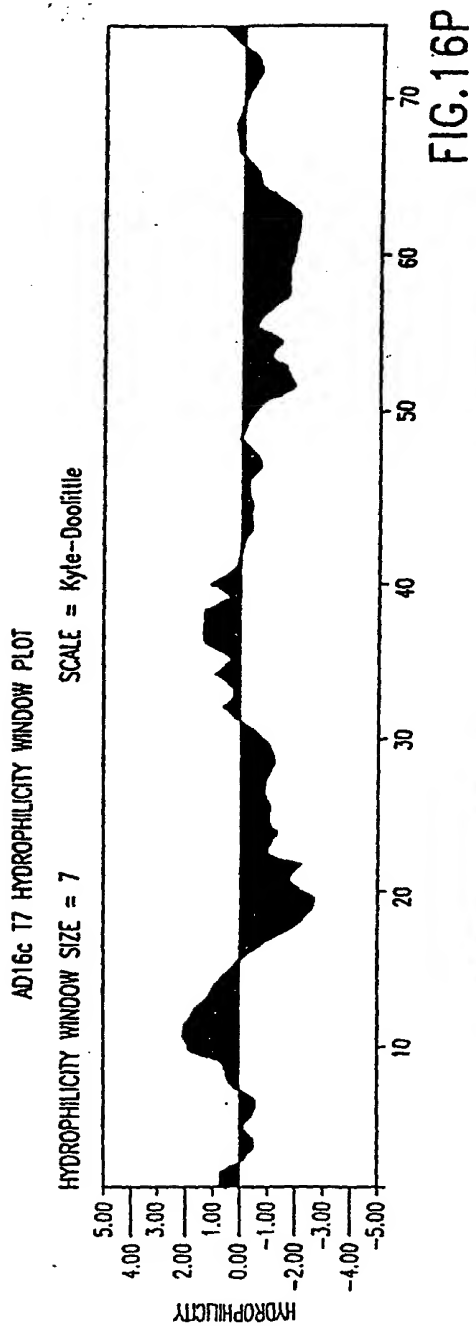


FIG.16P

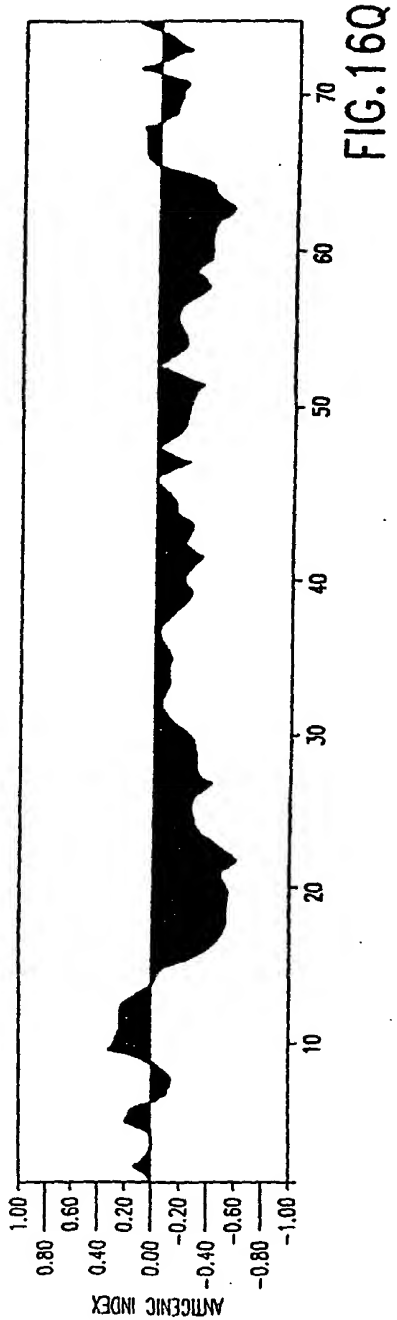


FIG.16Q

AD10-7 SEQUENCE

```
TTTTTTTTTT GAGATGGAGT TTTCGCTCTT GTTGCCCAGG CTGGAGTGCA ATGGCGCAAT  60
CTCAGCTCAC CGCAACCTCC GCCTCCCGGG TTCAAGCGAT TCTCCTGCCT CAGCCTCCCC 120
AGTAGCTGGG ATTACAGGCA TGTGCACCAC GCTCGGCTAA TTTTGTATTT TTTTTTAGTA 180
GAGATGGAGT TTAACTCCAT GTTGGTCAGG CTGGTCTCGA ACTCCCGACC TCAGATGATC 240
TCCCGTCTCG GCCTGCCCAA AGTGCTGAGA TTACAGGCAT GAGCCACCAT GCCCGGCCTC 300
TGCCTGGCTA ATTTTTGTGG TAGAAACAGG GTTTCACTGA TGTTGCCCAA GCTGGTCTCC 360
TGAGCTCAAG CAGTCCACCT GCCTCAGCCT CCCAAAGTGC TGGGATTACA GGCGTCAGCC 420
GTGCCTGGCC TTTTTATTTT ATTTTTTTTA AGACACAGGT GTACCACTCT TACCCAGGAT 480
GAAGTGCAGT GGTGTGATCA CAGCTCACTG CAGCCTTCAA CTCCTGAGAT CAAGCAATCC 540
TCCTGCCTCA GCCTCCCAAG TAGCTGGGAC CAAAGACATG CACCACTACA CCTGGTAATT 600
TTTATTTTTA TTTTTAATTT TTTGAGACAG AGTCTCACTC TGTCACCCAG GCTGGAGTGC 660
AGTGGCGCAA TCTTGGCTCA CTGCAACCTC TGCCTCCCGG GTTCAAGTTA TTCTCCTGCC 720
CCAGCCTCCT GAGTAGCTGG GACTACAGGC GCCCACCACG CCTAGCTAAT TTTTTTGTAT 780
TTTTAGTAGA GATGGGGTTT CACCATGTTC GCCAGGTTGA TCTTGATCTC TTGACCTTGT 840
GATCTGCCTG CCTCGGCCTA CCCAAAGTGC TGGGATTACA GGTCGTGACT CCACGCCGGC 900
CTATTTTTAA TTTTTGTTTG TTTGAAATGG AATCTCACTC TGTTACCCAG GTCGGAGTGC 960
AATGGCAAAT CTCGGCTACT CGCAACCTCT GCCTCCCGGG TCAAGCGATT CTCCTGTCTC 1020
AGCCTCCCAA GCAGCTGGGA TTACAGGACC TGCACCACAC CCGGCTAATT TTTGTATTTT 1080
CATTAGAGGC GGGTTTACCA TATTTGTCAG GCTGGGTCTC AAACTCCTGA CCTCAGGTGA 1140
CCCACCTGCC TCAGCCTTCC AAAGTGCTGG GATTACAGGC GTGAGCCACC TCACCCAGCC 1200
GGCTAATTTG GAATAAAAAA TATGTAGCAA TGGGGGTCTG CTATGTTGCC CAGGCTGGTC 1260
TCAAACTTCT GGCTTCAGTC AATCCTTCCA AATGAGCCAC AACACCCAGC CAGTCACATT 1320
TTTTAAACAG TTACATCTTT ATTTTAGTAT ACTAGAAAGT AATACAATAA ACATGTCAAA 1380
C                                                                 1381
```

FIG.16R

AD16c-SEQUENCE

CCATTGTTAG GTTGTCTCTT ACCTGTTAAA ATCAGGAGCT GACAAGAAAT GCTTACCACA 60
AAAGGAGAAA TGCCAGTCTA GTTAACAGTC AACGAGAGAA ATCAGGAAGA TTATGTGGGT 120
GGAAGAAGTA GATGATGTGG CTGATGAGTG AGTGAGTGAG CAAGCCTCCC CCCAGCTGAA 180
GAAGGAGTCA GAACTGCCCT TTGTTCCCAA CTATTTGGCG AACCCCAGCC TTCCCTTTTA 240
TCTATACACC CACAGCAGAG GATTCAGCCC AGATGCAGAA TGGGGCCCCC TCCACACCCC 300
CTGCATCACC CCCTGCAGAT GGCTCACCTC CATTGCTTCC CCCTGGGAAC CTCCCCTGTT 360
AGGGACCTTT CCCCGGGACC ACACCTCTTT GGCACTAGTT CAGAATGGTG ATGTGTCGGC 420
CCCTCTGCCA TACTAGAACA CCAGAAAGAC AAACGGGTGA TGTTTGTCAG CTACAGTGAG 480
TCTAGAGCCG TCCTGTTTTC TTCTGTCCCG TCCCAAGCCA CCATGTCTCT TCGAGCCTCA 540
AAATGGGACG TATGCAGGAC CAGCGCCCAG ATTCCAAGCC ATTTTTCTTC ACTGGAGCAT 600
TTCCATTTAA TATGCAAGAG CTGGTACTCA AGGTGAGAAT TCAGAACCCA TCTCTTCGAG 660
AAAATGATTT CATTGAAATT GAACTGGACC GACAGAGCTC ACCTACCAAG AGTTGCTCAG 720
AGTGTGTTGC TGTGAGCTGG GTGTTAATCC AGATCAAGTG GAGAAGATCA GAAAGTTACC 780
CAATACTCTG TTAAGGAAGG ACAAGGATGT TGCTCGACTC AAGATTTCAG GAGCTGGAAC 840
TGGTTCTGAT GATAGTGAAA ATAATTTTCT GTTCAGAAAT GCTGCATCAC ACTGACTGAA 900
AGGCCTTGCT ATACAGGAGA GCTTCAAAAC TGACTTACTA ATGCAGCAGG GACTTTTATA 960
CTGAGTATAT GACAGTGTGC ATCACCTCTG GGCCAAGGAC AAGCCATGAT CTAAATGCCT 1020
CAGATGCCCG GGCCAGTCTG GTGCACTGCA TAGTATATAC GAACATCATT CTGCCCAAGG 1080
TAGGAAGCCC CATGACCCCC AAGCAGTGGT GTCCACTCTT CCAAGCCTCT TGGTGCACAA 1140
TAAACCTTAT TGCTTGAAGC TTTGAACGAC TGTGAGAATG GTCTGGCGAG GACGAGAACG 1200
TGGAATTATA TGAGTGTCTT TTGTATCCGA GAATGTAGAG AGTTCTCTGA AGACGACGAC 1260
TGAGAGAGAG CGGACGCTAT TTCTAGCCAC TCCTGTTGAC AGTGCACCTG AAGGGCTGGG 1320
ATGCGTTTTT CTTGGTGTTG CATGCTCACA ACTCTGCTGA CATTGGGAAC TTATGAGAGA 1380
GGAAGACTCG GGAAAGCACA GATACTGGAC AGATGGATTC TGGTGTGGGG AAAGCACAGA 1440
TACTGGACAG ATGGTTCTAG TGTGACTTGT GACTGTGAGG TTTCCTATAA CATATTTATA 1500
AATGTTCATC AGGTTCAAAA GTCTATAAGA ATACAGTTCG AGACTGAATT GCTTCGAAAT 1560
ACTTCGATGT TGGGAACCAA AAGAGCTTTC CCTCCCTCAC TTTTTCCTTT GTAACACTCA 1620
TGACTGCTTC TCTGTCTCGA GTCATCTCTG CATTAACTCC CCTTCGTGGT CACTAGAGGG 1680
CTCTCTGATG CTTCTAAGAC ACTGCTTTTT ACATGCCACA CCCACCGCGT AGAGACAGGG 1740
TCTCACTATG TGGCCCAGGC TGGTCTCAAA CTTCTGGCCT TAAGTGATCG TCCTGTCCTT 1800
CGCGCTCGGA AGAAAGTCGT GGGGATTACA GGTGTGAGCC ACCGCGCCAG CCCCTCCCTT 1860
GTGTTTCAAC CAATCGGAAG TGAATTTAAC TAGATGTAGT AACCTTTTTT TTCTTTGACT 1920
TCTAAAAAAG TTACAGTTTA CTAATAAAGT TAAGTCTGGT TCTGTCCTAG AGGAAATAAA 1980
TTCACTATTA ATTCATGTCT TAAGTTACTT GGGTTAAAAC ACTTTCAGCC ACCCAGATTA 2040
ATTAAAGTGG AGCAGTGGAG CCCCTGGCTG GGGAGATGGG CCTCCAGAGG AGCAGCTGCA 2100
GGCATGTTCT GGCTACACAG AGGCAAGCAA GGGACTGGTG TCTCTGGTGA GAGGTGGGTT 2160
TGATGTATCT CTGTCCTATG CTGGTCTCTC TTCTCCTTTA TAAATCCTCC TGTGGTCACT 2220
GACTATCGTA TCGCAGTGAT CAGACTGCAC ATAGTACGGT TAGGCTGAGC TTAATGTCTT 2280
AATCATGTCA TTCGAGAGAA GACACGTTTT GATTCATGCT TTGTGTAATT AATCAATCAA 2340
GGATTCTTTT TTTAGCTTTG TTGACGTGTA ATTCACCCCT CCTCCTCCAC TGCATATTTA 2400
AAGCATGTGT TCACACTGTG TGTATACATT CACTGCGATT TTTTCGTTTG CTGCATTGCT 2460
TGGACTGTTC ATAACATCAC AAGTATTATT CAAATAAAAT ATTAACTGAC CGAAAAAAAA 2520

FIG.16S

```
H REG GENE                5   10   15   20   25   30   35   40   45   50
[ 220 ] ————————GA·AtTCC TGggC TCAaG TGATC CtCtC atgTC AGtCT CCCAA AGTGC TGGGA>
AD2-283           GA ACTCC TGACC TCAGG TGATC CCCCC GCCTC AGCCT CCCAA AGTGC TGGGA

H REG GENE  55   60    65        70   75   80        85       90   95   100
[ 220 ]     TgACA gGCtT G-AG- CC—A -CCAc ACcAg gcccA -TC— AtCa- G—tT ttTaT A-taA>
AD2-283     TTACA AGCGT GCAGA CCGCA CCCAG ACGAT TTTAA TTCTT AACNT GCCAT CCTCT ACCCA

H REG GENE   105   110   115   120   125  130   135   140
[ 220 ]     AGaAa AaAaa ACcTT AaaaT tgtTA gCAAA tacta tGACA>
AD2-283     AGTAC AGACC ACTTT AGTGT CCCTA TCAAA GGGGG AGACA
```

FIG.17

```
AD2 SP6F     110     115    120   125   130   135   140   145   150
[ 62 ]    ——AA ttC-t C-CtG cCTCA GCCtc gtGag ccGct GGgAT TACAG GcG>
EXONI       AA GCCAA CTCAG ACTCA GCCAA CAGGT AAGTG GCCAT TACAG GAG


RAT PTP   605
[ 144 ]   <ACTC
AD2-2 T7   ACTC

RAT PTP  660    655   650  645     640   635   630   625       620    615   610
[ 144 ]   <tcT-a ggaAg aGggg GTTGA C——t tTGCT TTTGA taGaT GGT-c TagT- TTCac TTttg
AD2-2 T7   AGTGT TTTAT TGCTA GTTGA CAAAG CTGCT TTTGA ATGCT GGTGG TTCTA TTCCT TTGAC

RAT PTP      710   705   700   695     690   685     680     675     670   665
[ 144 ]   <aCA-T aCAAt AaTGg aGatA -aaaa TaCcA T-A-G GgCAg T—GA GGcA- AgaaT GTTtG
AD2-2 T7   TCATT TCAAA ATTGC TGCCA GTGTT TTCAA TGATG GACAA TCAGA GGGAT ATGCT GTTAG

RAT PTP                                  745 740 735 730 725 720 715
[ 144 ]                                   <GTg A-Agg taTTT TTatT TaaaT gTgca gggTT
AD2-2 T7                                   GTA ATACT CCTTT TTTCT TCTTT CTTTT TTTTT
```

```
                                    5
          ————————————————————FVf cLII>
HPTPAA                          FVA SLIK
```

FIG.17A

```
1-9aT7-3 3  140   145   150  155   160   165   170   175   180   185   190   195
[ 206 ]   ACcAC GCCCc GCTAA TtTTT GTATT TTTAG TAGAG AcaGG GTTTC aCCgT GTTgg cCAGG>
AD2-283   ACAAC GCCCA GCTAA TATTT GTATT TTTAG TAGAG ATGGG GTTTC TCCAT GTTCA TCAGG

1-9a-T72 3   15    20    25    30    35    40    45    50    55    60    65    70
[ 260 ]   CTGGT cTgaA ALTCC TGggC TgAaG TGATC CtCCa GtCTt gGCCT CCCAA AGTGC TGGGA>
AD2-283   CTGGT GTCGA ACTCC TGACC TCAGG TGATC CGCCC GCCTC AGCCT CCCAA AGTGC TCGGA

1-9a-T72 3    140     145   150   155   160
[ 260 ]   AG-AC AcA-t A-Tag AtTGa gaC-A gaAAA>
AD2-283   AGTAC AGACC ACTTT AGTGT CCCTA TCAAA
```

FIG.17B

```
AD16c-SP6                                  5    10      15    20    25    30
[ 344 ]                              —AGA- TcTCg CTC-T G-Tca CCCAG GCTGa AGTGC>
AD2-2 SP6                             AGAG TTTCA CTCTT GCTTG CCCAG GCTGG AGTGC

AD2-2 SP6     35    40    45    50    55    60    65    70    75    80    85
[ 344 ]    AgTGG CcCAA TCtcG GCTCA CTGCg AgCTC C-aCC TCCCG gGtTC Actic aTTCT CCTGC>
AD2-2 SP6  AATGG CACAA TCCTG GCTCA CTGCA ACCTC CGCCC TCCCG AGCTC AAGAA CTTCT CCTGC

AD16c-SP6 100   105    110   115   120   125   130   135 140145   150  155   160
[ 344 ]    CTCAG CCTC- TGAGt aGCTG GGAcT ACAGG CGCcC aCCAC AcGCc gCTAA TtTTT GTATT>
AD2-2 SP6  CTCAG CCTCG TGAGC CGCTG GGATT ACAGG CGCGC GCCAC AAGCG ACTAA TATTT GTATT


AD16c-SP6
[ 344 ]    TTTGT AG>
AD2-2 SP6  TTTGT AG

AD16c-SP6     140   145   150  155   160   165   170   175   180   185   190   195
[ 206 ]    ACcAC GCCCc GCTAA TtTTT GTATT TTTAG TAGAG AcaGG GTTTC aCCgT GTTgg cCAGG>
AD2-283    ACAAC GCCCA GCTAA TATTT GTATT TTTAG TAGAG ATGGG GTTTC TCCAT GTTCA TCAGG

AD16c-SP6   200      205   210
[ 206 ]    aTGcT —CGA tCTCC TGA>
AD2-283    CTGGT GTCGA ACTCC TGA
```

FIG.17C

```
H REG GENE    3610  3615   3620  3625  3630    3635  3640 3645  3650  3655  3660
[ 118 ]       ——CC CC-aa gC-aG tGTta aTcct GG-cT A—CT GtgTG AGcTG AccTC AagcA CaGGt>
AD3-4           CC CCTGT TCTTG GGTGG GTTTC GGTAT ATTCT GGTTG AGATG ATATC ATTTA CGGGC

H REG GENE       3670 3675  3680 3685  3690 3695 3700 3705  3710 3715  3720
[ 118 ]       GAAGG Cagag aaTcc A-Tcc aCC-T gTTTC TgTTc TCCcT gCtTA gctcc AGGga TgGAA>
AD3-4         GAAGG CGCTT TGTGA AGTAG GGCTT ATTTC TCTTG TCCTT TCGTA CAGGG AGGAT TTGAA

H REG GENE  3725  3730  3735   3740  3745  3750   3755   3760  3765  3770
[ 118 ]       cTgGg Actgg GaT-a gAgga aaG-g TGAAC TC-cT CA-tT aaggg aATgG aTG>
AD3-4         GTAGT AGAAC GCTGT TACTC CGGTC TGAAC TCAGT CACGT GGCTT TATCG TTG
```

FIG.18

```
WP03-5 T7       5         15    20    25  30    35    40    45    50
[ 90 ]        GATCC aAGCT acGTA -CgcG TgcAT GCAcg tCaTa gcTcT TCTAT AGTGT CAC>
AD3-4 221     GATCC GAGCT CGGTA CCAAG TTGAT GCATA GCTTG AGTAT TCTAT AGTGT CAC

18-4T7       155   150   145       140   135  130   125   120   115   110   105
[ 362 ]      <gTATg GgCcc gATAg —c-t TAT-t TAgcC TTTAG AGCAC ACTGG CgGCC GTTAC TAGTG
AD3-4 221     ATATA GACAA TATAA CAATA TATTG TATAC TTTAG AGCAC ACTGG CAGCC GTTAC TAGTG

18-4T7       100   95    90    85   80    75    70    65    60    55    50    45
[ 362 ]      <GATCC GAGCT CGGTA CCAAc TTGAT GCATA GCTTG AGTAT TCTAT AGTGT CACct -aAaT
AD3-4 221     GATCC GAGCT CGGTA CCAAG TTGAT GCATA GCTTG AGTAT TCTAT AGTGT CACTA ATAGT
```

FIG.18A

```
G2A-EP T7                20   25   30   35       40   45   50        55
[ 148 ]    ----------CTT AaTA- gAtAg ctaCT TA--- AAAto AcTTA CaC-A cT--- GTtTt>
AD3-4 SPF                CTT ACTAC CAGAC AACCT TAGCC AAACC ATTTA CCCAA ATAAA GTATA

G2A-EP T7 60      65      70   75   80   85   90   95   100  105       110
[ 148 ]    aGaG- T-G--- cTTGA AAaCT atCtg AtcAG AcATA GTAtt GaAAc cAAtG A--At AcATT>
AD3-4 SPF  GGCGA TAGAA ATTGA AACCT GGCGC AATAG ATATA GTACC GCAAG GAAAG ATGAA AAATT

G2A-EP T7 115    120     125      130    135
[ 148 ]    AT-At aAAG- -TAA- A-gCa AAGGA -gAA>
AD3-4 SPF  ATAAC CAAGC ATAAT ATAGC AAGGA CTAA

AD3-4                          215  210  205      200  195  1901185  180
[ 182 ]    ----------------------<CT ATAaA GgTcG TTG--- TcaaC gaTaA AGCAc GtGAc
H REG GENE                      CT ATAGA GATTG TTGAT TTGCC TCTTA AGCAA GAGAT

AD3-4       175      170  165   160   155   150  145   140   135       125
[ 182 ]    <TgAgt tCAG- aCcGg A-Gta aCAG- CgttC TacTA CTTCA -aaTc cTC-C CTGCg aaAgG
H REG GENE  TCATT GCAGC TCAGC ATCGC TCAGA CCAGC TCATA CTTCA TGCTG ATCTC CTGCC TGATG

AD3-4      120  115  110  105   95    90   85   80   75   70   65   60
[ 182 ]    <caaga Gaaat AagGC CtAct TAAGc gC-CT TccCC cgtAA atgAt atcaT CTcaa CCaGA
H REG GENE  TTTCT GTCTC AGAGC CAAGG TAAGA TCTCT TTTCC ACCAA CCAAC TCTTT CTAGC CCTGA

AD3-4        55   50   45   40   35   30   25   20   15   10
[ 182 ]    <AtA-T aCcCa aaccC CCCAA GaAcA gGGGa ggAaa aGAAA AAAAA AAAAA
H REG GENE  AGACT TCACT CTATC CCCAA GCATA CGGGT CTACT TGAAA AAAAA AAAAA
```

FIG.18B

```
AD2-2 T7                    535  530  525   520  515  510  505  500  495
[ 110 ]   ----------<CAGAA AAcTa GCtAc aC-AA tgcAt TGGTC TATcA tgtta aAAcg
AD4-4 T7F                   CAGAA AAATT GCCAA GCTAA ACTAA TGGTC TATAA AAGCG TAATT

AD2-2 T7   490      485  480  475  470    465     460
[ 110 ]  <TGCAT -T--a GaCAc AAA-T ACAaA aacCA TG--A -AacA aGCcA
AD4-4 T7F  TGCAT GTGTG GGCAT AAACT ACAGA GCTCA TGCTA GAGTA TGCAA


1-9a                      145  140   135  130  125   120  115   110
[ 142 ]   ----------<AA -TTGg gTaC- -Cggg cccCc cCTA- gAggT cgAcG gTAT- cGA-T
AD4-4 T7F                 AA GTTGA TTGCT TCAAA TTTCT GCTAC TAACT TCAAG CTATG GGAGT

1-9a     105  100  95   90   85   80      75   70   65   60   55   50
[ 142 ]  <aaGct tGTAt cgAaT TccGG AcTTT gcTT- --TT gGTTT TCcTT Tcctg TGaaA AgGtT
AD4-4 T7F TTGGC AGTAG TCACT TGAGG ATTTT TTTTC CAATT CGTTT TCATT TTTGT TGTTA AAGCT

1-9a        45   40    35   30   25    20   15   10
[ 142 ]  <gGTtt TaaAG TGAG- -AtAc ActTT tCC-- GtogA ACaAg TGtTC TAT
AD4-4 T7F  CGTAC TTCAG TGAGA CAGAA AAATT GCCAA GCTAA ACTAA TGGTC TAT
```

FIG.19

```
H REG GENE                      5   10   15   20   25   30   35   40  45
[  278 ]————————————————g AATTC CTGGG CTcAA GTGAT CCTCt coTgT coGtC TCCCA AAGTG>
AD16C-T7-A                A AATTC CTGGG CTGAA GTGAT CCTCC AGTCT TGCCC TCCCA AAGTG

H REG GENE   50   55   60   65   70   75  80   85   90   95  100   105
[  278 ]   CTGGG ATgAC AGGCt TGAGC cACco coCCo gcCCo TcATc AgTtt TToTA tAooG -oooo>
AD16C-T7-A CTGGG ATTAC AGGCA TGAGC TACTG AGCCT AGCCT TAATG ATTAA TTTTA GAGTG ATGGC

H REG GENE  110   115      120  125   130      140  145  150   155  160
[ 278 ]    ooooA CCT-t A—oA Attgt TA-gG cooAt AotGA cAAoT TgtAo to-to tottc ttACo>
AD16C-T7-A TTGTA CCTTC AAGCA ACATA TAGAG TTGAC ACAGA AAATT TCCAT CGTCC CGAGA AAACT

H REG GENE 165   170  175  180  185 190  195  200  205  210  215  220
[ 278 ]    tTtCo -gATt tttot ttTtT aooCT GotAA GAott gotTA oTAAo TAoAA tTtoG tAT—>
AD16C-T7-A GTGCT GCATG GGCCC CGTGT GCCCT GTGAA GATCC CCCTA TTAAC TATAA ATGGC CATTG

H REG GENE       225   230  235
[ 278 ]    t-oA- -tcTG t-ctt TToA>
AD16C-T7-A CACAT GGTTG CCAGC TTCA
```

FIG.20

```
AD16C                                          5    10    15    20
[ 266 ]  ——————————————————————————————————aACgt TtcGA aCCTa tCgTg>
Human-PTP                                      TACTG TGTGA GCCTG ACCTC

AD16C          25    30    35    40    45        50    55    60    65    70
[ 266 ]  AAG-c -ccGA TTttA GAgtT aatAc -ggGT -gC— tTcAA GggA- acggg gCTaT —ga->
Human-PTP AAGCA CAGGA TTCCA GAAAT GGAAG GATGT GCCTT GTGAA GACAA GTTCT CCTTT GTCTG

AD16C        75      80    85       90    95   100   105   110115120   125   130
[ 266 ]  gAAGT -tttc tACgg GgaGC —aTG GAAAt TttcT GTCTc aAtaT GtgCt tGaAg gTACA>
Human-PTP CAAGT TCAAA AACTA GAGGC ACCTG GAAAA TACAT GTCTA GAACT GATCC AGCAA TTACA

AD16C     135  140   145   150   155    160   165   170   175 180     185   190
[ 266 ]  ACcGt aTCtA AAATT AAtCa tt-aa ggCTa ggCtc agtAg CTCtg CCTGt -a-aT CcCag>
Human-PTP ACGGA GTCAA AAATT AAACC GGACC ATCTC TCCAA CTCAA CTCAA CCTGG ACACT CTCTT

AD16C       195   200   205       210   215   220   225    230   235    240
[ 266 ]  CaC-t tTcgG gagGC Caa— gAcTg gaggA TcacT TcAg- ccCag gAa-t TTcaG AcgCc>
Human-PTP CTCTG CTGAG TTTGC CTTGT TAATC TTCAA TAGTT TTACC TACCC CAGTC TTTGG AACCT

AD16C-T7-A                                     205
[ 33 ]   ——————————————————————————————————VPCE Dr>
HPTPAA                                         VPCE DK

RPTP AA   115   120
[ 33 ]     SgSLf LyKsW D>
Translatio SSSLG LPKCW D
```

FIG.20A

ALIGNMENT OF AD16C-SP6 cDNA WITH AD2-2 SP6 cDNA

```
AD2-2 SP6   40    45        50    55    60    65    70    75    80    85    90
 [ 362 ]  CTtGC T-TG- —CCC AGGCT GgAGT GCAaT GGCaC AATCc tGGCT CACTG CaAcC TCCcC>
AD16C-SP6- CTCGC TCTGT CACCC AGGCT GAAGT GCAGT GGCCC AATCT CGGCT CACTG CGAGC TCCAC

AD2-2 SP6 95   100   105   110   115   120     125   130       135   140   145
 [ 362 ]  CTCCC GaGcT CAaga acTTC TCCTG CCTCA —GCC TC-G- ——TG AGccG CTGGG AtTAC>
AD16C-SP6- CTCCC GGGTT CACTT CATTC TCCTG CCTCA CTGCC TCAGC CTCTG AGTAG CTGGG ACTAC

AD2-2 SP6  150   155   160       165   170   175   180   185
 [ 362 ]  AGGCG CgCgC CA-CA aG—— -CgaC TAA-T aTTTG TATTT TT-GT AG>
AD16C-SP6- AGGCG CCCAC CACCA CGTCC CCTGC TAATT TTTTG TATTT TTAGT AG

AD2-283     50    55    60    65    70    75    80  85    90    95   100   105
 [ 374 ]  CCaTG TTcat CAGGc TGGTg TCGAa CTCCT GACCT CGTGA TCCGC CCGCC TcaGC CtCCC>
AD16C-SP6- CCGTG TTGGC CAGGA TGGTC TCGAT CTCCT GACCT CGTGA TCCGC CCGCC TTGGC CACCC

AD2-283  110   115   120   125   130
 [ 374 ]  AAAGt G-cTG GGATT ACAaG CGTGC>
AD16C-SP6- AAAGA GTTTG GGATT ACAGG CGTGC
```

FIG.20B

FIG. 21A

FIG. 21B

FIG. 21C

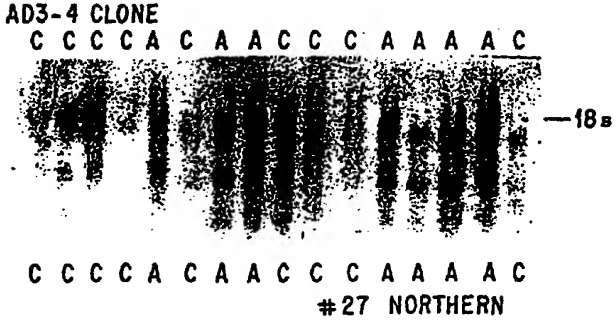


FIG. 21D

G2-2Pst-M13F SEQUENCE

Sequence Range: 1 to 251

TGCAG CAATG GCAAC AACGT CTGCA AACTA TTAAC TGGCG AACTA CTTAC TCTAG CTTCC 60
CGGCA ACAAT TAATA GACTG GATGG AGGCG GATAA AGTTG CAGGA CCACT TCTGC GCTCG 120
GCCCT TCCGG CTGGC TGGTT TATTG CTGAT AAATC TGGAG CCGGT CGAGC GTGGG TCTCG 180
CGTAT CATTC GAGCA CTGGC GCCAG ATGGT AAGCC CTCCG TATCG TAGTT ATCTC ACAGC 240
AGGGA GTCAG G 251

FIG.22

G2-2Pst-M13R SEQUENCE

Sequence Range: 1 to 242

TGCAG GAGCG GGGAG GCACG ATGGC CGCTT TGGTC CGGAT CTTTG TGAGG AACCT TACTT 60
CTGTG GTGTG ACATA ATTGG ACAAA CTACC TACAG AGATT TAAAG CTCTA AGGAA ATATA 120
AAATT TTTAA GTGTA TAATG TGTTA AACTA CTGAT TCTAA TTGTT TGTGT ATTTT AGATT 180
CCAAC CCTAT GGAAC CTGAT GAATG GGAGC CAGTG GTGGA ATGCC TTTAA TGAGG AAACC 240
TG 242

FIG.22A

G2-2Pst1-EcoR1-M13F SEQUENCE

Sequence Range: 1 to 208

TGCAG CAATC TTTCT TATAT ACATG CTTAA TAGAT AGCTA CTTAA AATAA CTTAC ACACG 60
TTTTA GAGTT GCTTG AAAAC TATCT GATCA AGACA TAGTA ATTGA AACCA ATGAA TACAT 120
TATAT AAAGT AAAGG AAAGG AGAAG AGAGC AAAGG GAGGG GAAGA GGAGA GGGAG GGACA 180
AGCGA GAAAG GAAAG GGAAG GGAGA AAA 208

FIG.22B

Gen2-2Pst1-EcoR1-M13R SEQUENCE

Sequence Range: 1 to 152

CTCAC TAAAG GGATC AAGGA ATAAT TTTGA ATTTC AAGTC TTACA TTTAA TAAAT ACATT 60
CATAA GGCTA TAACT ACCAT ACGTT GTGAT TTCTC TGATT AATTT AAAAA TAAAT TAAAA 120
CCTGG AAAGA ATTTT ACCAT TCTAG GAAGC CA 152

FIG.22C

G2-2Pst1-EcoR1-T7 SEQUENCE

Sequence Range: 1 to 338

AATCT ATCTT ATATA CATGC TTAAT AGATA GCTAC TTAAA ATAAC TTACA CACGT TTTAG 60
AGTTG CTTGA AAACT ATCTG ATCAA GACAT AGTAA TTGAA ACCAA TGAAT ACATT ATATA 120
AAGTA AAGGA AAGGA GAAGA GAGGA AAGGA GGGGA GAGGA GAGGA GGACA AGCGA GAAAA 180
CGAAC GGAAG GGAGA AAAAG GGGGA AAGGG AGGTA GAGAG AGAGA GAAAA AGTGC TGGTC 240
ATATA GTAAG TGTAC ATTTT AACTT TTTAA GAAAC TACCC TACTC TATTC CAGAG TGATT 300
GTACA TGTGC ATTTT ACTGC ATTAT AGAGA TCATT TTC 338

FIG.22D

G5dPst-M13R SEQUENCE

Sequence Range: 1 to 169

TGCAG GAGTG GGGAG GCACG ATGGC CGCTT TGGTC CGGAT CTTTG TGAAG GAACC TTACT 60
TCTGT GTGTG ACATA ATTGG ACAAA CTACC TACAG AGATT TAAAC GTCTA AGGTA AATAT 120
AAAAT TTTTA GTGTA TAGGT TAAAC TACTG ATTCT AATGT TGTGT ATTT 169

FIG.22E

G5d Pst-T71 SEQUENCE

Sequence Range: 1 to 209

CCCCG GGCTG CAGCA ATGGC AACAA CGTCT GCAAA CTATT AACTG GCGAA CTCAT TCATC 60
TAGCT TCCCG GCAAC AATTA ATGAC TGGAT GGAGG CGGAT AAAGT TGCAG GACCA CTTCT 120
CGCGT GGCCC TTCCG GCTGG CTGGT TTATT GCTGA TAATT GAGCG TGCGA GTGGC TCGCG 180
TATCA TTCGC GACAT GGGCC AGTAG GTAC 209

FIG.22F

G5dPst1-EcoR1-SP SEQUENCE

Sequence Range: 1 to 272

CTTGC CCTTC ATGGA GTCAT ACAGC CGATC AGCAA AATGC AGGGG CTTGT TCTGA ATGCA 60
CTGAA CCAGG TTCAG GAAAG CATTT TCCAG GTCTC CTTTA ACCTC TTTCC TGATG CTTTC 120
CAACA TGTCA TAAGG GCTGT AACTC TTGTA CCTAT CAAAT ACTTT CTGGA GGTGG GGACA 180
CGCTC CGCTC GGTCA TGATG CTGAT CCACT TGGGA ACATC AGTTC TTTCC TCTTC ACTCC 240
AGCTG CATAG AGATC CGAGG ACTCT TGGTC AA 272

FIG.22G

G5dPst1-EcoR1-t7 SEQUENCE

Sequence Range: 1 to 278

ACGGC CCAGC TTCCT TCAAA ATGTC TACTG TTCAC GAAAT CCTGT GCAAG CTCAG CTTGG 60
AGGGT GATCA CTCTA CACCC CCAAG TGCAT ATGGG TCTGT CAAAG CCTAT ACTAA CTTTG 120
ATGCT GAGCG GGATG CTTTG AACAT TGAAA CAGCC ATCAA GACCA AAGGT GTGGA TGAGG 180
TCACC ATTGT CAACA TTTTG ACCAA CCGCA GCAAT GACAC GAGAC AGGAT ATTGC CTTCG 240
CCTAC CAGAG AAGGA CCAAA AAAGG AACTT GCATC ACA 278

FIG.22H

ALIGNMENT OF G2-2Pst1 with HUMAN REG GENE (1)

```
H REG GENE     3405 3410     3415 3420 3425  3430 3435   3440 3445 3450 3455
[ 228 ]        ——AG CAATa GCAA- -AgGa aaGgA AAC-A aTAtt TaGC- AA-gg tTTAt TCTtc CTTtg>
G2-2Pst-M13F   AG CAATG GCAAC AACGT CTGCA AACTA TTAAC TGGCG AACTA CTTAC TCTAG CTTCC

H REG GENE          34653470 3475 3480 3485 3490 3495  3500   3505 3510
[ 228 ]        tGtCA gCAtT TctgA GtgTG cAcac AGGCc cAgtg A-TTc CAtG- -tAtT TtTGa G-T-G>
G2-2Pst-M13F   CGGCA ACAAT TAATA GACTG GATGG AGGCG GATAA AGTTG CAGGA CCACT TCTGC GCTCG

H REG GEN3515  3520 35253530  3535 3540   3550 3555 3563565    3575 3580
[ 228 ]        aCCac TgCct CTGtC TGG-c ccTTc CccAT AgAaC cGccG CtGGT gGAGC GTGGG TCcCt>
G2-2Pst-M13F   GCCCT TCCGG CTGGC TGGTT TATTG CTGAT AAATC TGGAG CCGGT CGAGC GTGGG TCTCG

EXON                                          20   25     30    35   40
[ 124 ]        ————————————————————————————C TGGca CtGG- aG-ca GTGGG TCcCt>
G2-2Pst-N13F                                  C TGGAG CCGGT CGAGC GTGGG TCTCG

H REG GENE     3585 3590 3595 3600   3605  3610 3615   3620  3625 3630
[ 228 ]        gGTcT CcTaC aAGtc CTGGG G-CA- tTGG- -AGCC CcaaG cA—G T-GTT A-aTC ctgGC>
G2-2Pst-M13F   CGTAT CATTC GAG Q CTGGG GCCAG ATGGT AAGCC CTCCG TATCG TAGTT ATCTC ACAGC

EXON5          45   50   55   60   65   70     75   80     85   90   95
[ 124 ]        gGTcT CcTaC aAGtc CTGGG G-CA- tTGG- -AGCC CcaaG cA—G T-GTT A-aTC ctgGC>
G2-2Pst-M13F   CGTAT CATTC GAG Q CTGGG GCCAG ATGGT AAGCC CTCCG TATCG TAGTT ATCTC ACAGC

H REG GENE3635  3640
[ 228 ]        ActGt GTgAG>
G2-2Pst-M13F   AGGGA GTCAG

EXON5          100  105
[ 124 ]        ActGt GTgAG>
G2-2Pst-Mo3F   AGGGA GTCAG
```

FIG.23

ALIGNMENT OF G2-2Pst with HUMAN REG GENE (2)

```
H REG GENE   3155 3160 3165  3170 3175 3180 3185 3195       3200
[ 194 ]  ——AG GAGac ttGtG GtA-a AaatC tGCTg ctGTa CtGcT CaTT- TG-GG AACCT TA-gT>
G2-2Pst-M13R AG GAGCC GGGAG GCACG ATGGC CGCTT TGGTC CGGAT CTTTG TGAGG AACCT TACTT

H REG GEN3210  3215 3220 3225  3230 3235 3240 3245 3250 3255 3260  3265
[ 194 ]     aTact aaaTa AtATA A-Tat AtcAA CaACt aAtgG tcAgc cAAtG CTaTg ctG-g ATATg>
G2-2Pst-M13RCTGTG GTGTG ACATA ATTGG ACAAA CTACC TACAG AGATT TAAAG CTCTA AGGAA ATATA

H REG GENE 3270  3275  3280 3285 3290 3295  3300 3305   3310 3315 3320
[ 194 ]     AgggT ccT-g GgccA cAAaG acaaA AAaT- CaGga aCcAc TT-TT TaaGT gagaT ActTT>
G2-2Pst-M13RAAATT TTTAA GTGTA TAATG TGTTA AACTA CTGAT TCTAA TTGTT TGTGT ATTTT AGATT

H REG GEN3325  3330  3335 3340 3345 3350 3355 3360 3365 3370 3375
[ 194 ]     gggtC tCTgT -cAAa tTcAT aAcac ttAtt tctTG GTGGA ATaCa gTTAA TGAG>
G2-2Pst-M13RCCAAC CCTAT GGAAC CTGAT GAATG GGAGC CAGTG GTGGA ATGCC TTTAA TGAG


G2-2Pst-Ma3F     150  155  160      165  170   175  180  185  190  195
[ 130 ]       ———TA aAtCt GgaGC cGG— -TcGA GC-GT GGGTC tCgcG TaTCa TtCgA GcaCT>
EXON5            TA GAACC GCCGC TGGCA CTGGA GCAGT GGGTC CCTGG TCTCC TACAA GTCCT

G2-2pst-M13F 200    205    210  215  220  225  230   235  240  245  250
[ 130 ]      GGGGC —caG A-tgg tAAGC ccTcc gtATC gTaGt TA-Tc TcacA GCagG gagTC AgG>
EXON5        GGGGC ATTGG AGCCC CAAGC AGTGT TAATC CTGGC TACTG TGTGA GCCTG ACCTC AAG
```

FIG.23A

ALIGNMENT OF G2-2PST-ECOR1-T7 WITH HUMAN REG GENE

```
H REG GENE                                335  340  345  350  355  360  365
[ 132 ]  ————————————————————AT AGATA tCTAC TTtAt tcgAt TTAaA ttCTG -TTTA>
G2-2PST-ECOR1 T7             AT AGATA GCTAC TTAAA ATAAC TTACA CACTG TTTTA

H REG GENE    370  375  380  385       390  395  400  405  410  415
[ 132 ]   —GTa tTTtA ttA-T ATtTt gTtA- A—T- ccAtT TG-tc CCAAT tcATA tAcT- TAT>
G2A-EP T7 GAGTG CTTGA AAACT ATCTG ATCAG ACATA GTAAT TGAAA CCAAT GAATA CATTA TAT

RAT PTP                                                          740  735
[  82 ]  ————————————————————————————————————————————————————<TqAAq qtATT
G2-2PST-ECOR1-M1                                                  TAAAT
ACATT

RAT PTP          730   725   720   715      710  705  700  695  690  685
[ 82 ]    <—Ttt attTA -AAtg tgCA- ggGTT -ocA- TaCaa TaATg gAgaT AAAAA TAcca TAggg
Gen2aEP-Ma  CATAA GGCTA TAACT ACCAT ACGTT GTGAT TTCTC TGATT AATTT AAAAA TAAAT TAAAA

RAT PTP          68675  670  665       660  655
[ 82 ]            <CgaGG cAAGA ATgTT ——tg TCTAG GAAG
G2-2PST-ECOR1-M1  CCTGG AAAGA ATTTT ACCAT TCTAG GAAG
```

FIG.23B

```
Human-PTP          270        280  285   290   295   300   305    310     315
[ 166 ]     ——— AcCTG GtGct gTgCT cAC-C cAGgc cgaGG gtgC- cTTtg TgGcC T-cAc tGAtt>
G5dPst-t           AACTG GCGAA CTACT TACTC TAGCT TCCGG CAACA ATTAA TAGAC TGGAT GGAGG

Human-PTP 320    325   330 335   340   345   350   355         360   365   370
[ 166 ]     aaG-g AgAGT gGCAt GAtgA CTTCa atG-T CtG-g aTT— GGC— -CTcc aTgAc ccCaa>
G5dPst-t    CGGAT AAAGT TGCAG GACCA CTTCT GCGCT CGGCC CTTCC GGCTG GCTGG TTTAT TGCTG

Human-PTP  375  380  385   395 400   405   410   415   420   425   430    435
[ 166 ]    AaAgA aCcGc cGCtG GgGAG CGTGG GTCcC tgGTc TCcTa caAGt cCTGG GG-CA -tTGG>
G5dPst-t   ATAAA TCTGG AGCCG GTGAC CGTGG GTCTC GCGTA TCATT GCAGC ACTGG GGCCA GATGG

Human-PTP       440   445     450   455   460   465   470
[ 166 ]    —AGC CCcaa GcA— GTGtT aATCc tggCt ACtGt GtG-A -GcC>
G5dPst-t   TAAGC CCTCC GTATC GTGGT TATCT ACACG ACGGG GAGTA CGGC
```

FIG.23C

```
H REG GENE                          80    85   90       95  100 105  110  115
[ 158 ]  ---------- AG GC-Cc ATcat CagTT T--T- tatAT aaaga aaAAa aAACC TTAaa>
G5dPst-M                AG GCACG ATGGC CGCTT TGGTC CGGaT CTTTG TGAAG GAACC TTACT

H REG GENE  120  125  130  135  140  145  150  155  160  165  170      175
[ 158 ]      atTGT taGgc AaATA cTatG ACAAA -Ttgt aAtAt AtATT cttAC aTtTc AGaT- -tTtT>
G5dPst-M     TCTGT GTGTG ACATA ATTGC ACAAA CTACC TACAG AGATT TAAAC GTCTA AGGTA AATAT

H REG GENE  180  185  190 1195  200  205  210  215  220
[ 158 ]  AtttT TTaaA cTGTA TAGaa TtgAt TAaTa AaTaa AAT-T T-aGT ATT>
G5dPst-M AAAAT TTTTA GTGTA TAGGT TAAAC TACTG ATTCT AATGT TGTGT ATT

G5dPst-t                125   130  135       140  145   150  155  160  165
[ 118 ]  ----------TA aAtCt GgaGC cGG-- -T-GA GC-GT GGGTC tCgcG TaTCa TtgcA GcaCT>
EXON5                   TA GAACC GCCGC TGGCA CTGGA GCAGT GGGTC CCTGG TCTCC TACAA GTCCT

G5dPst-t  170     175   180  185  190  195  200     205       210  215  220
[ 118 ]    GGGGC --caG A-tgg tAAGC ccTcc gtATC gTGGt TA-Tc T-acA --cG ACggg gAGtA>
EXON5      GGGGC ATTGG AGCCC CAAGC AGTGT TAATC CTGGC TACTG TGTGA GCCTG ACCTC AAGCA

G5dPst-t
[ 118 ]    C-GG>
EXON5      CAGG
```

FIG.23D

FIG. 24A

FIG. 24B

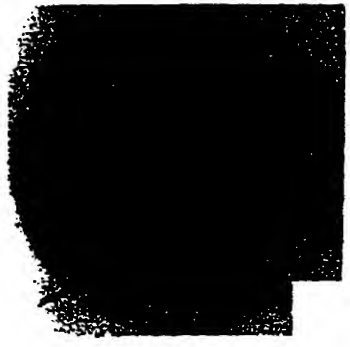


FIG. 24C

FIG. 24D

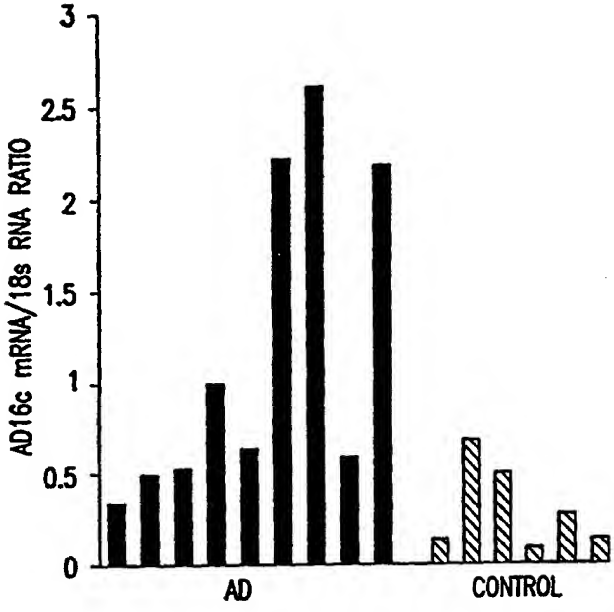


FIG.25A

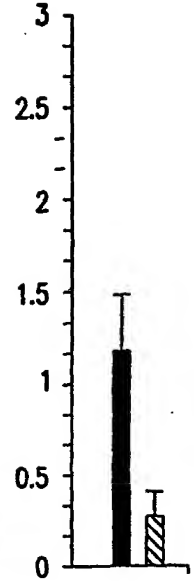


FIG.25B

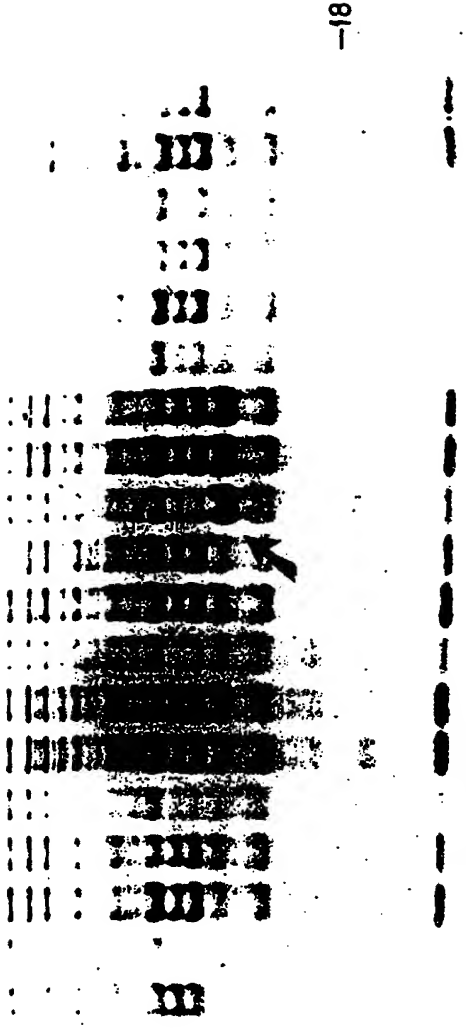


FIG.26

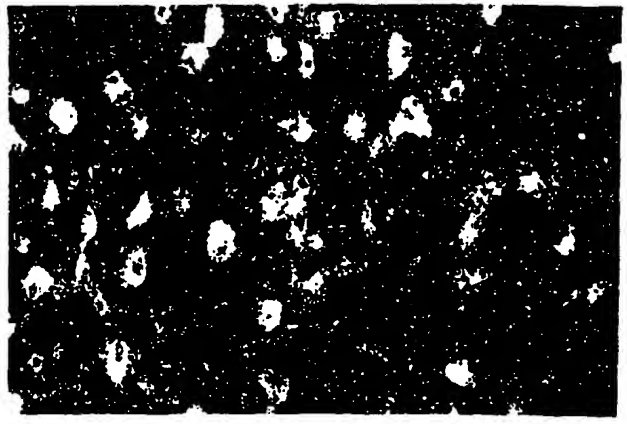


FIG. 27A

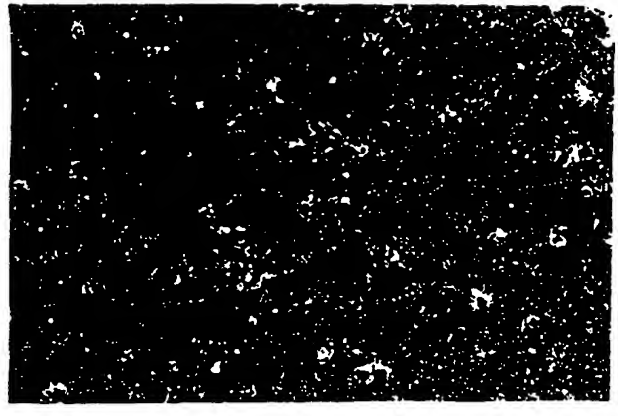


FIG. 27B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/17111

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 69.1, 240.1, 320.1; 424/9.1; 530/350+; 536/23.5, 24.31, 24.5; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	American Journal of Clinical Pathology. Volume 100, issued July 1993, M. Abe et al, "Production of Immunodiagnostic Applications of Antihuman Light Chain Monoclonal Antibodies", pages 67-74, see especially page 74, paragraph 1.	1-20
X	Science, Volume 237, issued 03 July 1987, S. Bahmanyar et al, "Localization of Amyloid β Protein Messenger RNA in Brains from Patients with Alzheimer's Disease", pages 77-80, see especially page 79.	30-31, 37, 41 and 44-48

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 15 MARCH 1996	Date of mailing of the international search report 25 MAR 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Deborah Crouch, Ph.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/17111

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Nucleic Acids Research, V lume 22, N . 12, issued 1994, R.B. Denman et al, "Ribozyme Mediated Degradation of β-Amyloid Peptide Precursor mRNA in COS-7 Cells", pages 2375-2382, see especially pages 2380-2381, bridging paragraph.	38-40,42 and 43
X	Nature, Volume 331, issued 11 February 1988, P. Ponte et al, "A New A4 Amyloid mRNA Contains a Domain Homologous to Serine Proteinase Inhibitors", pages 525-527, see especially figure 2, page 526.	21-29,33, 36, 49 and 50

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/17111

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12Q 1/68; G01N 33/53; C12P 21/06; C12N 5/00, 15/00; A61K 31/70, 49/00; C07K 1/00; C07H 21/02, 21/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 7.1, 69.1, 240.1, 320.1; 424/9.1; 530/350+; 536/23.5, 24.31, 24.5; 514/44